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Abstract

Oats (*Avena spp.*) is a cereal crop mainly used in food and feed applications. Common oats (*Avena sativa*) has gained attention because of health supporting properties and potential in reducing the risk of chronic diseases, related to its bioactive compounds β-glucan, tocopherols, tocotrienols and avenanthramides. The interest for other species has increased as they may be valuable in food applications due to their contents. Content variations in common oats is well known, but fairly unknown in other species. Previous research showed interesting properties of one relative, *Avena strigosa* which was investigated in the present study. Variations in the contents of β-glucan, tocopherols, tocotrienols and avenanthramides as well as the molecular weight distribution of β-glucan were studied among thirteen variety samples. Samples from two varieties were subjected to false malting (i.e. exclusion of sprouting) to evaluate effects. There was a significant difference among the samples regarding all studied bioactive compounds ($p=0.000$). The β-glucan content ranged between 1.9 and 6.3 % (w/w) and the average molecular weight between 1.59×10^6 and 2.43×10^6 g/mol. Among studied E-vitamers α-tocotrienol was the highest and varied between 7.4 and 60.0 µg/g DM. Other E-vitamers (α-tocopherol, β-tocotrienol and β-tocopherol) varied between 5.1 and 15.9, 1.3 and 7.2, 0.8 and 3.6 µg/g DM respectively. The main avenanthramides 2c, 2p, 2f and 2fd ranged between 11 and 352, 19 and 211, 7 and 435, from below detection limits to 112 nmol/g DM respectively. The main hydroxycinnamic acid was caffeic acid which varied between 17 and 134 nmol/g DM. The β-glucan content, the average molecular weights of β-glucan, the contents of avenanthramides and hydroxycinnamic acids were affected by false malting, while the contents of E-vitamers were unchanged. Generally, malting lead to a decrease in the content and in the average molecular weight of β-glucan, but caused a pronounced increase in mainly the lately eluted avenanthramides, 2pd and 2fd, especially in samples from one of the two varieties. Even though this study found similarities when comparing *A. strigosa* and *A. sativa* certain features were different. The importance of these differences can only be evaluated in human studies which investigate the differences in bioactivity. Further studies are needed to fully evaluate the variation among varieties of *A. strigosa* and to get a realistic picture of its true potential as a novel oat food crop. Factors to consider should though be the contents of several bioactive compounds and also the responses after processing like malting since the responses may differ between samples. *A. strigosa* is well worth to investigate further according to the present results.

Keywords: Oats, *Avena strigosa*, β-glucan, tocopherols, tocotrienols, avenanthramides, steeping, false malting, variety sample variation.

Sammanfattning

Havre (*Avena spp.*) är en spannmålsgröda som främst används som livsmedel och foder. Havre (*Avena sativa*) har uppmärksammats p.g.a. dess hälsobefrämjande egenskaper och potential att minska risken för kroniska sjukdomar relaterade till dess bioaktiva ämnen, speciellt β-glukaner, tokoferoler, tokotrienoler och avenantramider. Nyligen har även andra arter av havre uppmärksammats eftersom de möjligen kan vara värdefulla i livsmedel p.g.a. de höga halterna av hälsobefrämjande ämnen. Variation av de bioaktiva ämnena inom *A. sativa* är välkänt, medan variation inom andra havrearter är betydligt mindre studerat. Tidigare forskning visade intressanta egenskaper hos havrearten *A. strigosa* som undersöktes i denna studie. Variation av halterna av β-glukaner, tokoferoler, tokotrienoler och avenantramider samt β-glukanernas molekylviktsfördelning studerades i prover från tretton olika sorter. Prover från två sorter studerades även efter ”falsk groning” (dvs. groning som inhiberar utväxt av grodd) för att utvärdera effekterna. Studien visade en signifikant skillnad mellan proverna avseende de bioaktiva ämnena ($p=0.000$). β-Glukanhalten varierade mellan 1.9 och 6.3 % och medelmolekylvikten hos β-glukan mellan 1.59 och 2.43×10^6 g/mol. Bland de undersökta E-vitamererna var halten av α-tokotrienol högst och varierade mellan 7.4 och 60.0 µg/g TS. Andra E-vitamerer (α-tokoferol, β-tokotrienol och β-tokoferol) varierade mellan 5.1 och 15.9, 1.3 och 7.2 samt 0.8 och 3.6 µg/g TS. De dominerande avenantramiderna (2c, 2p, 2f och 2fd) varierade mellan 11 och 352 nmol/g TS, 19 och 211, 7 och 435 samt från under detektionsgränsen till 112 nmol/g TS. Den mest förekommande kanelsyran var kaffesyra som varierade mellan 17 och 134 nmol/g TS. β-Glukanhalten, medelmolekylvikten av β-glukaner, halterna av avenantramider och kanelsyror påverkades av falsk groning. Generellt sett, orsakade groningen en minskning av β-glukanhalten och medelmolekylvikten av β-glukan, medan halterna av E-vitamerer var oförändrade. Den falska groningen ledde däremot till en uttalad ökning av halterna av de sent eluerade avenantramiderna, 2pd och 2fd, främst i prover från en av de två sorterna. Även om denna studie fann likheter då *A. strigosa* jämfördes med *A. sativa*, så noterades även skillnader. Huruvida dessa skillnader är av betydelse eller ej kan bara fastställas genom humanstudier som utvärderar skillnader i bioaktivitet. Ytterligare studier behövs för att utvärdera sortvariationen inom *A. strigosa* så att en realistisk bild av dess potential att användas som en livsmedelsgröda kan tydliggöras. Att ta i beaktande är halterna av flera olika bioaktiva ämnen, men också effekterna av processer som groning eftersom både halterna och effekterna kan variera mellan olika prover. *A. strigosa* är väl värd att undersöka ytterligare enligt resultaten i denna studie.

Nyckelord: Havre, *Avena strigosa*, β-glukan, tokoferoler, tokotrienoler, avenantramider, blötläggning, falsk groning, sortvariation inom analyser.

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Abbreviations

AVA	Avenanthramide
AVAs	Avenanthramides
DM	Dry matter
DON	Deoxynivalenol
GOPOD	Glucose oxidase/peroxidase reagent
HCAs	Hydroxycinnamic acids
HPLC	High Performance Liquid Chromatography
HT-2	Type A-trichothecene
NSP	Nonstarch polysaccharides
M_{cf}	Calcofluor average molecular weight
mw	Molecular weight
T2	Type A-trichothecene
α-TP	α-Tocopherol
β-TP	β-Tocopherol
α-TT3	α-Tocotrienol
β-TT3	β-Tocotrienol

1 Introduction

The common oat (*Avena sativa*) is a cereal crop and a member of the *Pocaceae* family. It is grown worldwide and used in multiple ways, e.g. as food, feed and as an ingredient in skin health products. As food products, oats is mainly used as an ingredient in porridges, breakfast cereals, cookies and in snack bars (Strychar, 2011; Webster, 2011). In addition, oats is used to produce dairy substitution products (Coultate, 2009). In the past years the health benefits from oat consumption have been highlighted due to the nutritive value provided especially from mixed-linkage (1→3)(1→4)-β-D-glucan (hereafter referred to as β-glucan), tocopherols and avenanthramides (AVAs). The non-starch and indigestible fibre component β-glucan is known to lower blood cholesterol and to decrease the risk of cardiovascular disease (Mathews, 2011), while specific forms of tocopherols constitute the essential vitamin E. Tocopherols and tocotrienols are fat-soluble antioxidants (Coultate, 2009). Avenanthramides, the unique bioactive phytochemicals of oats, may provide health benefits by their anti-inflammatory activities (Ji *et al.,* 2003*;* Liu *et al.,* 2004; Nie *et al.,* 2005) and also by their function as antioxidants (Bratt *et al.,* 2003; Jastrebova *et al.,* 2006). Previous studies have shown significant variations among oat cultivars regarding the content and mw distribution of β-glucan, the content of major E-vitamers and AVAs (Bryngelsson *et al.,* 2002a; Jastrebova *et al.,* 2006; Andersson & Börjesdotter, 2011). Therefore health effects may vary between cultivars of common oats. Breeding towards varieties with increased contents is therefore motivated. Another way to go is the use of processing methods, e.g. malting, which may increase the content of bioactive compounds. Alternatively, oat varieties with higher amounts may be specifically selected for food applications.

Since the ancient art of beer brewing was invented the process of malting kernels has been performed by mankind. It is commonly known that germination increases the contents of many nutrients like proteins, fats and phenolic compounds in kernels (Tian *et al.,* 2010; Donkor *et al.,* 2012). Previous research shows that the amount of AVAs is increased by malting of oat kernels (Bryngelsson *et al.,* 2003;

Skoglund *et al.,* 2008; Skoglund, 2008; Collins & Burrows, 2012). One malting method which increases the content of AVAs is "false malting", where anaerobic steeping is included (Collins & Burrows, 2012). However, studies have shown that the content and molecular weight of β-glucan is decreased during specific germination methods (Wilhelmson *et al.,* 2001; Doehlert & McMullen, 2003; Hübner *et al.,* 2010). Certain technologies have therefore been studied and tailored to maintain the β-glucan content to a higher extent throughout malting in barley by changing pH, time and temperature (Rimsten *et al.,* 2002; Haraldsson *et al.,* 2004). The possibility to tailor a germination method which retains the β-glucan content and mw distribution simultaneously as it increase the AVA content, is yet to be evaluated.

Other species of oats can be found, e.g. *A. byzantina* (red oat), *A. strigosa* (bristle or sand oat) and *A. abyssinica* (Ethiopian oat). In addition, there are several wild relatives to the commonly grown *A. sativa*. Recently especially *A. strigosa* has shown interesting properties like high contents of β-glucan, tocopherols, tocotrienols and AVAs among many different analyzed species in the AVEQ project (*Avena Genetic Resources for Quality in Human Consumption*) (Germeier *et al.,* 2011; Dimberg, personal communication, 2012). Historically *A. strigosa* has mainly been used as feed, cover crop or has even been regarded as a weed but shows a pronounced adaptability to scarce environments (Kuszewska & Korniak, 2009; Germeier *et al.,* 2011).

Today little is known about the variation of health promoting bioactive substances like β-glucan, tocopherols, tocotrienols and AVAs in grains among varieties of *A. strigosa*. Knowledge about the variations in common cultivars of *A. sativa* is substantially wider in comparison and has been studied since the 1980s.

1.1 Aim

This study aimed to explore the variations among thirteen different samples of *A. strigosa* varieties regarding the content and molecular weight of β-glucan, and the content of tocopherols, tocotrienols and AVAs. Samples from two varieties of *A. strigosa* were also subjected to a malting process similar to "false malting" (Collins & Burrows, 2012) to explore the possibility to increase the bioactive compounds of interest and to still retain a satisfactory amount and molecular weight distribution of β-glucan.

2 Literature study

Literature was studied through screening of scientific databases provided by the library of the Swedish University of Agricultural Science, Uppsala. The databases *Web of knowledge*, *Scopus* and *Pubmed* were used to find relevant articles in the field from 1990 until present time. Keywords like "Avenanthramide* AND beta-glucan* AND oat* OR "*Avena* sativa" AND toco*" were used to find comprehensive scientific articles. Literature searches for specific authors were in addition done and literature was also found through citations in articles. Furthermore, archives in the library in Uppsala were used to locate specific publications from scientific journals. The supervisors were also contributing with some relevant references and method descriptions.

2.1 Oats (Avena spp.)

Oats (*Avena spp.*) is a cereal crop and is grown in widespread locations. It is known to be a relatively hardy crop in farming systems, but is also threatened by sequela (Strychar, 2011; Valentine *et al.,* 2011). Oats are harvested with the hulls still attached to the caryopsis and it is estimated that the hulls makes up to about 25 % of the total weight. The term "groats" is used to describe the dehulled caryopsis or "grain". An oat specific botanical character is the hairlike trichomes, which are structures attached to the groat. Similar to other cereals, the botanical structures of the oat groat are pericarp, seed coat, nucellar epidermis, germ and endosperm. The aleurone layer is the outermost botanical cell layer of the endosperm (Delcour & Hoseney, 2010).

The commonly cultivated *Avena sativa* is used in many different ways, but mainly as feed and food ingredients. In food applications it is almost exclusively consumed as whole grains (whole caryopsis), where it has been mechanically dehulled and heat treated with steam for 1 h reaching a temperature of ca 90 ºC and later further processed to rolled or instant-cooking oats. The heat treatment aids in inactivation of putrefying enzymes like lipases, peroxidases and lipoxygenases

which otherwise may cause rancidity and development of unwanted flavors. In addition, β-glucan decomposing enzymes, β-glucanases, becomes inactivated upon the heat treatment. Furthermore, it will also lead to the development of typical sensory properties associated with oat products, but will inevitably affect other functional properties such as the solubility of proteins (Bryngelsson *et al.*, 2002b; Delcour & Hoseney, 2010; Webster, 2011).

There are several commercial oat products available, e.g. rolled oats (wholegrain flakes), oat flour and oat bran. Food products derived from oats are divided into hot cereals and cold cereals where hot cereals basically include rolled or instant oat flakes, generally recognized as breakfast cereals. Cold cereals include granola and muesli, where whole-oat flakes and oat bran are the main ingredients. Oat products may be added as ingredients in bakery products, infant foods, soup and sauce thickeners, pancake mixtures and in meat extenders. One other novel product is the β-glucan enriched oat bran which is highly interesting because of the health supporting role associated with β-glucan consumption (Webster, 2011). In recent years, oat products have also been used as dairy product substitutions, for example oat drinks, because of the absence of lactose (Coultate, 2009).

As a feed, oats is mainly added in cereal-based feed mixtures and given to cattle because of its high protein content. Oat-based feeds were very common before the mechanization of agriculture, due to the large number of workhorses. Today, oat-based feeds are still popular for race or hobby horses (Strychar, 2011). The entire oat crop may also be harvested as hay and silage (Valentine *et al.*, 2011)

Different oat varieties occurs and are grown worldwide, although some of them are not used or cultivated in a wide commercial range. In fact, some are even considered to be weeds (Kuszewska & Korniak, 2009; Germeier *et al.*, 2011). Oat varieties are gathered in the *Avena spp.* and occur in different chromosome numbers. In the European countries cultivated hexaploid oats are the common *A. sativa* and the closely related *A. byantina*, also called red oats. The diploid *A. strigosa* and the tetraploid Ethiopian oat *A. abyssinica* belong to marginally cultivated oats in the European countries. Some hexaploid wild relatives of *Avena spp.* are e.g. *A. fatua*, *A.sterilis* and *A. hybrid*. Other wild species are e.g. *A. hirtula* (diploid), *A. wiestii* (diploid) and *A. barbata* (tetraploid) (Germeier *et al.*, 2011).

The nutritive composition of oat differs depending on species, cultivar and environmental factors that prevail. In one recent study different species were involved in an impartial screening that aimed to find species suitable for food applications and for breeding in that respect. The study found interesting properties for especially *A. strigosa*. Knowledge about the nutritive composition of marginally used varieties is today limited and there is hope that they may prove to be valuable for both human and cattle as foods and feeds (Germeier *et al.*, 2011).

The oat species *A. strigosa* (sand or bristle oat) is a diploid member of the *Avena spp.* family. Traditionally, its main use has been as a fodder and cover crop in agricultural systems, because of its adaptability to scarce nutrient environments and because of its relatively low fungal infection tendency. It has mainly been cultivated in European countries like Great Britain, Germany, Switzerland, Portugal, Spain and Poland. Little is known about the nutritive composition of *A. strigosa*, but has been reported to be characterized by higher contents of proteins, fats and sugars compared to common oats (reviewed by Kuszewska & Korniak, 2009).

Although the range of applications is broad, oat production is not especially big-scaled compared to cereal production worldwide. In fact, it was listed after wheat, maize, rice, barley and sorghum in a review by Brennan & Cleary (2005) and also by Welch (2011). One realistic explanation to this, at least in the Nordic countries, may be the increasing contents of mycotoxins like DON, T2 and HT-2 produced by various *Fusarium spp.* for example by *F. graminearum* and *F. langsethiae* (Langseth & Rundberget, 1999; Børnstad & Skinnes, 2008). On a bigger scale oat cultivation is threatened by crown rust infections caused by *Puccinia coronate* which is considered to be the main pathogen of oats globally (Rines *et al.*, 2007; reviewed by Park, 2008). Mycotoxins lead to poor safety and quality of cereal products both in food and feed. Comprehensive studies have investigated the factors which increase the risk of mycotoxin production and the picture seems to be complex as several conditions may induce mycotoxin production in co-dependent systems. Factors along the entire cereal production chain, from cultivation to processing (e.g. dehulling) may affect the amount of mycotoxin in cereal products (Langseth & Rundberget, 1999; Hormdork *et al.*, 2000; Børnstad & Skinnes, 2008). Varieties which show a greater resistance against these fungal infections are therefore desirable (Chen *et al.*, 2007; Valentine *et al.*, 2011).

2.1.1 Nutritive composition of oats

In *Table 1* the nutritive composition of oatmeal is described together with corresponding values of some other cereal crops. Generally, oats is similar to wheat, rye and barley in amounts of carbohydrates and ash. The major component of cereal grains is starch (Welsh, 2011). Normally the protein content of oats is higher compared to wheat, rye and barley. In addition, the nutritive amino acid composition is more advantageous in oats (Biel *et al.*, 2009). A difference between these cereals is the higher amount of fat in oats compared to wheat, rye and barley which may explain the high energy value of oatmeal (*Table 1*). In addition, the fatty acid composition of oats is comparatively low in polyunsaturated fatty acids like linoleic acid (18:2) and linolenic acid (18:3), but higher in the proportion of oleic acid (18:1) (Youngs,1986; Welch, 2011).

The soluble nonstarch polysaccharide fraction of oatmeal is together with the corresponding value of wholegrain rye the highest among cereals (*Table 1*). Oatmeal and pearled barley show remarkable amounts of β-glucan which is the main component of the soluble nonstarch polysaccharide fraction (Welch, 2011), whereas arabinoxylans are dominant in rye (Delcour & Hoseney, 2010). The amount of β-glucan in oats is known to vary and contents between 2 to 8 g/100 g of oat groats have been reported (Andersson & Börjesdotter, 2011; Welch, 2011).

Oat contains slightly higher amounts of major and minor minerals compared to other cereals. Oatmeal is in addition fairly high in vitamin E (α-tocopherol), biotin, thiamin, riboflavin, pantothenic acid and folic acid. On the other hand, oatmeal contributes with lower amounts of niacin and vitamin B_6 in comparison to wheat, rye and barley (Welch, 2011).

Apart from these nutritive compounds oats also contain other components which probably also contribute to the maintenance of health functions, e.g. phenolic compounds (Collins, 2011).

Table 1. The nutritive composition of oatmeal compared to some cereals expressed in values per 100 g (modified after Welch, 2011)

	Oat (Wholegrain)	**Wheat (wholegrain)**	**Rye (wholegrain)**	**Barley (pearled)**
Water (g)	**8.5**	**12.0**	**13**	**10.3**
Carbohydrate (g)	**58.7**	**60.2**	**58.7**	**69.7**
Dietary fibre (g)	9.0	10.6	12.8	8.0
Soluble NSP (g) (% tot.)	4.5 (58)	2.3 (22)	4.5 (35)	3.9 (33)
Tot. β-glucan (g)	4.4	0.83	2.07	4.2
Protein (g)	**14.0**	**13.5**	**11.2**	**9.2**
Fat (g)	**8.0**	**2.1**	**2.3**	**1.6**
Ash (g)	**1.8**	**1.6**	**2.0**	**1.2**
Energy (kJ)	**1473**	**1270**	**1215**	**1331**

2.1.2 Health claims concerning oat consumption

The legislation concerning health claims of food products varies between countries, but reasons and purposes are consistent worldwide - to improve consumer health status in both developed and developing countries. Today, health claims concerning oat and/or wholegrain consumption are published within the European Union, United States and in Malaysia (Mathews, 2011).

During the last 30 years health benefits derived from oat consumption has been highlighted mainly due to the hypocholesterolemic properties of oats, i. e. the cholesterol-lowering character. However, novel science suggests yet further reasons behind the health benefits related to the protection against cardiovascular disease. Consumption of oat containing products may affect blood glucose levels, blood pressure and body weight. In addition, there is a growing agreement that an in-

creased consumption of wholegrain products may protect against coronary heart disease, obesity, diabetes and some cancers (Mathews, 2011).

The EU Commission Regulation (432/2012) claims that β-glucan contributes to the maintenance of normal blood cholesterol levels. The health claim can be used for foods which contain at least 1 g β-glucan from oats, oat bran, barley, barley bran or from mixtures of these cereals per quantified portion. The beneficial effects are obtained when the daily intake reaches 3 g of β-glucan from these cereal sources. Another health claim related to β-glucan is the reduction of blood glucose levels after a meal when oat or barley derived β-glucans are consumed. Foods are then required to contain at least 4 g β-glucan from oats or barley for each 30 g available carbohydrates in a quantified portion as part of the meal. Oat grain fibre is furthermore claimed to contribute to an increase in faecal bulk.

Vitamin E is claimed to contribute against oxidative stresses of cells according to the EU Commission Regulation (432/2012). However, the food product must qualify as a source of vitamin E accordingly to the legislation.

At present time there is no health claim linked to AVAs since very few human studies have been performed.

2.1.3 β-Glucans

β-Glucan is found in all cereals, but the highest amounts are encountered in oats and barley (Wood, 2011). The chemical structure is a chain of repetitive glucose units linked through β-(1→3) and β-(1→4) glycosidic linkages, where the β-(1→4) linkages are the main bonds (*Figure 1*) (Coultate, 2009).

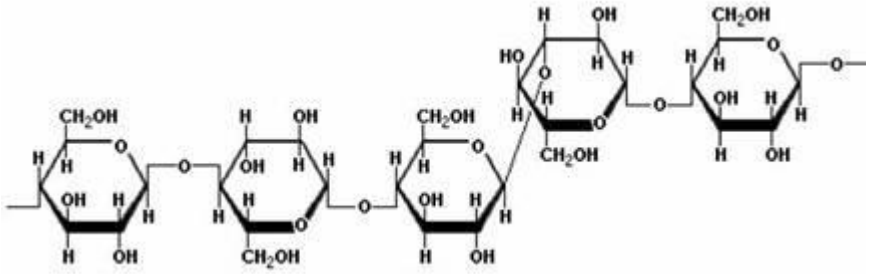

Figure 1. Basic structure of the β-glucan polymer chain (Havrlentová *et al.*, 2011).

It is a viscous partly water soluble polysaccharide that is mainly found in cell walls of starchy endosperm cells (Wood, 2011). In oats, the subaleurone layer is especially high in β-glucan (Miller & Fulcher, 2011). Furthermore, it is present in smaller amounts in the aleurone layer, probably also in the germ, leave and stem of oats. In contrast, β-glucan is not found in the hull of oat kernels. The amount of β-glucan in oat groats is commonly known to vary and values from 1.8 % up to 8.5

% have been reported. Differences are most likely attributed to both genetic and environmental factors (Andersson & Börjesdotter, 2011; Wood, 2011).

β-Glucan exists in a rather wide range of molecular weights (mw), as do other polysaccharide polymers. The distribution of mw will affect the rheological properties of any polysaccharide. The solubility or extractability and viscosity are characterized by the mw and the structure. When in contact with water, β-glucans with high mw possesses random coil structures leading to high viscosity at low concentrations. When depolymerized, β-glucan changes in conformation and may start gelation. β-Glucan with lower mw will start gelation quicker than if the mw is high. Generally, the oat β-glucan has higher mw compared to barley β-glucan. Both oat and barley β-glucan are mainly composed of β-(1→3) linked cellotriosyl and cellotetraosyl units. It is known that the amount of β-(1→3) linked cellotriosyl units affects the gelation character. In barley there is a higher proportion of cellotetraosyl units compared to in oat β-glucan. This may lead to different gelation properties of oats and barley (Wood, 2011).

There are convincing evidence that β-glucan provide health effects through its cholesterol-lowering character, which in a longer perspective leads to a reduced risk of heart disease. These beneficial properties are likely attributed to the high viscosity of β-glucan which increases the re-synthesis of bile acids with cholesterol as a precursor compound in the small intestine. The consequence is a higher cholesterol turnover and a lower cholesterol level in the blood. In addition, the low Glycaemic Index of oat products, due to an entrapment of available starch, may also add to the health benefits provided from oat consumption (Coultate, 2009).

2.1.4 Antioxidants

Antioxidants are radical scavenging compounds i.e. are reacting with free radicals in biological systems to prevent oxidation processes (Coultate, 2009). These functions may be evident in food products such as prevention of rancidity reactions, in color preservation, in development of flavor character and in keeping of texture (Madhavi *et al.*, 1996). In living animals and humans antioxidants may contribute in the defense against heart disease, certain cancers and in membrane damaging according to a review by Halliwell (1999).

Cereal flour, e.g. oat flour, is rather prone to oxidation due to the relatively high lipid content and due to the finely milled texture. Oats contain antioxidant compounds which protects the fatty acids from oxidative reactions. These are essentially E-vitamers (tocopherols and tocotrienols), free and bound phenolic acids and AVAs according to a review by Peterson (2001). One might therefore suspect a relationship between the content of lipids and antioxidants in oats, but according to Bryngelsson *et al.* (2002a) there could be no such general conclusion. However, they found high E-vitamer contents (especially tocotrienols) in both groats and

hulls of oats which also had high total lipid contents. The authors suggest that the lack of consistency in the codependence between antioxidants such as AVAs and lipids propose functions other than antioxidant properties of oat phenolic compounds (Bryngelsson *et al.*, 2002a).

Processing of oat products may affect the antioxidants. During processing, cellular structures are affected which may influence the availability and contents of oat antioxidants. Some bound antioxidants may be released as an effect of processing (Peterson, 1995; Peterson, 2001; Bryngelsson *et al.*, 2002b).

2.1.4.1 Tocopherols and tocotrienols

E-vitamers (tocopherols and tocotrienols) occur in plants and mainly in oils due to the fat soluble character. The chemical backbone constitutes a chromanol-ring linked to a saturated phytyl-side chain in tocopherols, whereas the side chain is unsaturated in tocotrienols (*Figure 2*). Both tocopherols and tocotrienols occur as α-, β-, γ- and δ-forms. The methylation of the chroman ring decides the form. All substances are found, even though only α-, β- and γ-TP and α- and β-TT3 are common (Coultate, 2009).

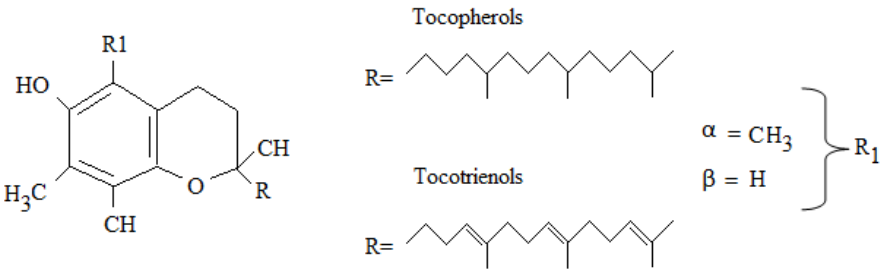

Figure 2. Chemical structure of tocopherols and tocotrienols (after Bryngelsson *et al.*, 2002b).

Individual forms also have specific properties. The essential vitamin E for example is mainly related to the activity of α-TP, even if other forms also tend to show activity. Among the tocotrienols α-TT3 provides the highest vitamin E activity (Coultate, 2009). The proposed function of vitamin E in the body is to stabilize cell membranes against oxidation in different tissues. It is believed that vitamin E may protect LDL cholesterol from oxidation and thereby protect against heart disease. In addition, the functions of vitamin E are required to maintain a normal immune defense (Nilsson *et al.*, 2008).

Tocopherols easily break down during heat treatments (Peterson, 1995; Peterson, 2001). In addition, Bryngelsson *et al.* (2002b) found that individual tocopherols and tocotrienols were affected in different ways by steaming of oat grains and

groats. They suggested that the increase in mainly the β-TP content during heat treatments may be due to a concurrent release of other bound E-vitamers and due to other compounds which co-eluted with β-TP during analysis. However, the release of other E-vitamers may be more unlikely as an explanation compared to the possibility that other compounds were co-eluting with β-TP. One explanation proposed by the authors is the different occurrence of E-vitamers within the kernel. In oats, tocopherol levels are highest in the germ, while tocotrienols mainly prevail in the starchy endosperm, although α-TT3 also occurs in high amounts in the bran. Drum drying of oat grains and groats on the contrary, caused decreases in E-vitamers. This was probably caused by enzymatic and hydrolytic reactions caused by the processing conditions (Peterson, 1995; Bryngelsson *et al.,* 2002b).

2.1.4.2 Avenanthramides and hydroxycinnamic acids

Avenanthramides are oat specific phenolic compounds. The chemical backbone constitutes specific anthranilic acids and hydroxycinnamic acids joined together through amide linkages. The combination determines the character of individual AVAs and there are ca 30 different AVAs identified (Bratt *et al.,* 2003; Jastrebova *et al.,* 2006; Dimberg, personal communication, 2012). Dependent on involved anthranilic and hydroxycinnamic acids, AVAs are grouped in five categories. For example, group 2 consists of 5-hydroxy-anthranilic acids (2) and the hydroxycinnamic acids caffeic-, *p*-coumaric- and ferulic acid (c, p and f) (*Figure 3*). The terminology is not entirely consistent when labeling AVAs. Even so, one easily understood way of tracking components is the use of following abbreviations for group 2: 2c, 2p and 2f. These are known to be the major AVAs in oats (Bratt *et al.,* 2003; Jastrebova *et al.,* 2006). In addition, also dienes of 2p and 2f have been found which are AVAs with an extra double bond in the hydroxycinnamic acid molecule. These are abbreviated as 2pd and 2fd (Dimberg, personal communication, 2012). Sinapic acid has not yet been found to occur in an AVA compound, although free sinapic acid is found in oats (Emmons & Peterson, 1999; Dimberg, personal communication, 2012). It is known that the different hydroxycinnamic acids possess different antioxidant activity (Dimberg *et al.,* 1993; Bratt *et al.,* 2003). Bratt *et al.* (2003) reported a slightly higher antioxidant activity of AVAs in category 2 in comparison to those AVAs derived from category 1, suggesting that also the anthranilic acids shows effects. These results implicated a structure-antioxidant activity relationship and it is therefore motivated to study the contents of different forms of AVAs.

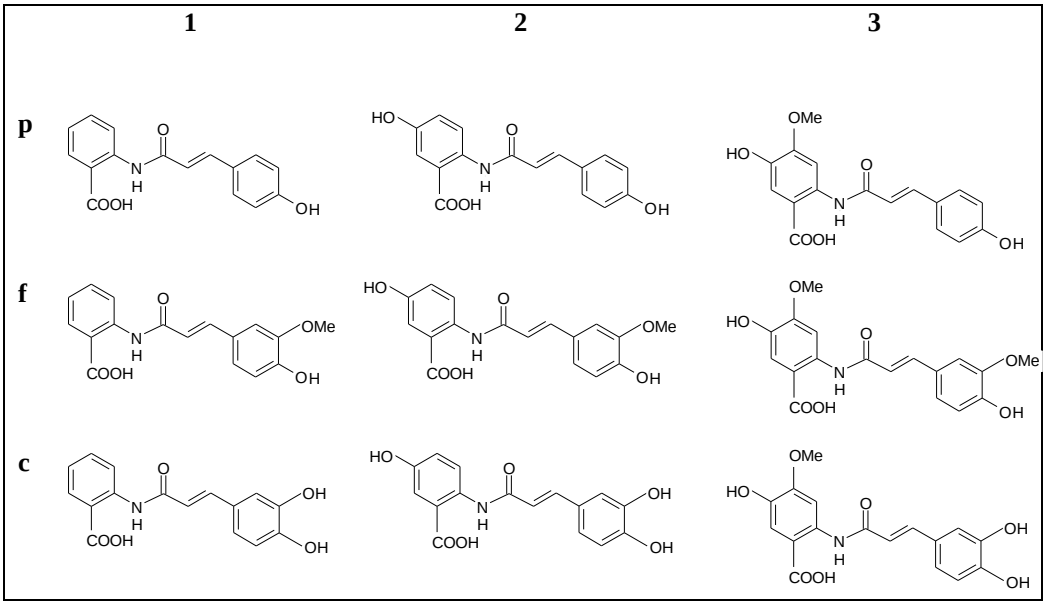

Figure 3. The chemical backbone of avenanthramides in group 1, 2 and 3. The left ring structure represents anthranilic acids: anthranilic acid (1), 5-hydroxy anthranilic acid (2) or 5-hydroxy-4-methoxyanthranilic acid (3). The right ring structure represents hydroxycinnamic acids: caffeic acid (c), *p*-coumaric acid (p) or ferulic acid (f).

Avenanthramides are present in groats, hulls, leaves and spikelets of oat plants (Dimberg *et al.*, 1993; Bryngelsson *et al.*, 2002a; Jastrebova *et al.*, 2006; Peterson & Dimberg, 2008). Avenanthramides are mainly located in the oat bran of groats (Dimberg *et al.*, 1993; Jastrebova *et al.*, 2006). They are, apart from antioxidants, also believed to function as phytoalexins i.e. are produced in plants as response to external stress e.g. fungal challenges by incompatible races of fungi (Mayama *et al.*, 1981; Mayama *et al.*, 1982; Bryngelsson *et al.*, 2002a). Avenanthramides probably also contribute to the flavor of oats. In one study by Molteberg *et al.* (1996) a connection between the perceived typical oat flavors was connected with a high content of AVAs. In humans, avenanthramides are believed to have anti-inflammatory properties and may provide protective functions against cardiovascular diseases and allergies (Ji *et al.*, 2003*;* Liu *et al.*, 2004; Nie *et al.*, 2005).

The content of AVAs in oats varies from a few mg kg^{-1} up to above 100 mg kg^{-1} (Dimberg *et al.*, 1993; Dimberg *et al.*, 1996; Molteberg *et al.*, 1996; Bryngelsson *et al.*, 2002a; Bratt *et al.*, 2003; Mattila *et al.*, 2005). Previous science has shown that the amount of AVAs increases during plant development and are present in spikelets at 3-5 days after heading (the prior step to anthesis) (Peterson & Dimberg, 2008). It was also shown that as the total accumulation of AVAs in spikelets increased, 2c was the main AVA when grain moved towards maturity. In contrast, the main AVA in leaves was 2p and no correlation was established between leave- and spikelet concentrations of AVAs among samples. The same study reports sig-

nificantly different amounts of 2c, 2p and 2f in developing oats of different genotypes analyzed after different harvest times. In addition, the composition and relative amount of AVAs changes during plant development, suggesting that the initial predominant form may not be the most evident in the final grain. As implicated by the authors, this can be related to their prevalence in the kernel. Early developed AVAs are mainly located in the empty glumes, *lemma* and *palea*, while the later detected ones are synthesized in the groat (Peterson & Dimberg, 2008). The synthesis of AVAs is performed by the hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase (HHT) enzyme according to Ishihara *et al.* (1999). Matsukawa *et al.* (2000) was able to detect HHT activity in the endosperm and scutellum of oat seeds. Peterson & Dimberg (2008) found that the HHT activity was evident in spikelets already 21-29 days after heading in different genotypes. As a consequence, the HHT activity may be connected to the amount of AVAs during plant development. Yet, another consideration was the presence of AVAs even before any HHT activity was recorded in the study. This indicates that other factors are probably also of concern regarding the occurrence of AVAs (Peterson & Dimberg, 2008).

Studies have not been able to detect an established correlation regarding the relationship between contents of AVAs and degree of rust infection, although there is some evidence. Some studies show that the contents were increased when plants were stressed and infected by fungus, while other shows more vague connections (Mannerstedt-Fogelfors, 2001; Peterson *et al.*, 2005). The HHT enzyme seems to be activated by the exposure of pathogen substances like mycotoxins (Ishihara *et al.*, 1997). Nevertheless, probably variations due to genotypic and environmental factors play a major role. The cropping system might therefore be of concern. Dependent on the use of fungicides, crop rotation strategies and soil preparation etc. the infection risk can differ in organic and conventional cropping systems. Whether these factors affect AVA contents are yet to be evaluated. One 3-year study though concluded that there was no difference between oats from organic systems compared to conventional systems in terms of their AVA amounts (Dimberg *et al.*, 2005).

Apart from the hydroxycinnamic acids (HCAs) that are recognized in oat-specific AVAs (c, p and f), there are also other conjugates present. These may be bound to a solid matrix, can be present in conjugated forms and may exist in free forms. Together they constitute the main part of phenolic compounds in cereal groats (Adom & Liu, 2002). Mostly HCAs are present in bound forms according to a review by Faulds & Williamson (1999). Dimberg *et al.* (2001) reported the finding of another oat-specific HCA compound, a lignan which is esterified to a sucrose molecule, also known as truxinic acid sucrose ester (TASE). Bryngelsson *et al.* (2002a) found that oat hulls contain AVAs and HCAs, but TASE were not

detected. Oats differ widely in their contents of HCAs and TASE due to genetic factors (Dimberg *et al.,* 1996; Dimberg *et al.,* 2005; Peterson *et al.,* 2005; Shewry *et al.,* 2008).

2.1.5 Effects of germination on β-glucans, E-vitamers and avenanthramides

According to Lehtinen & Kaukovirta-Norja (2011) the term germination refers to the transformation of biological storage compounds within grains into building blocks of new plants. Furthermore, malting is described as a controlled version of germination. Generally, the nutritive value of cereals is changed as a response to germination. In oats the contents of protein, fat, reducing sugars and starch were increased upon germination (Donkor *et al.,* 2012). The content of starch was though decreased in a study by Tian *et al.* (2010), where also changes in amino acid composition were noted and the phytate content (an anti-nutritional compound) was decreased. In addition, several phenolic compounds are known to increase, possibly due to the improved extractability gained during germination (Donkor *et al.,* 2012; Tian *et al.* 2010).

Germination of cereal products may lead to improved texture and flavor properties (Wilhelmson *et al.,* 2001), as steeping and germination soften the kernel structure and changes the chemical composition. Sprouting and long time germination of oats however, leads to loss of β-glucan content, decrease in molecular weight and subsequent cell degradation (Wilhelmson *et al.,* 2001; Doehlert & McMullen, 2003; Hübner *et al.,* 2010). Damages due to sprouting may also be induced in the field when grain maturity is reached and there are high precipitations before harvest (Doehlert & McMullen, 2003). A suggestion in literature is that specific germination methods which induce seeds in dormancy may inhibit subsequent germination stages and sprouting of kernels (Collins & Burrows, 2012). This may be valuable strategies for an improved retainment of β-glucan.

There is not much documentation regarding germination effects on E-vitamers in oats. However, Haraldsson *et al.* (2004) reported an increase in tocopherols and tocotrienols when samples of barley were steeped in 15 ºC compared to when steeped in a higher temperature of 48 ºC.

Controlled and adapted steeping and germination methods have been reported to increase the content of AVAs (Bryngelsson *et al.,* 2003; Skoglund *et al.,* 2008; Collins & Burrows, 2012) in oats along with an increased HHT activity (Bryngelsson *et al.,* 2003; Skoglund *et al.,* 2008). They showed that the method parameters such as time and temperature should be considered (Skoglund *et al.,* 2008). One further consideration is that the germination temperatures should be kept low to avoid growth of moulds and hence circumvent mycotoxin production as far as possible (Wilhelmson *et al.,* 2001).

3 Material and Methods

3.1 Oat sample analysis

3.1.1 Oat samples

Thirteen different oat samples of *Avena strigosa* (*Table 2*, Appendix *Figure 4-7*) with different origin, cultivation regimes, years and locality were supplied by Maria Scholten. Samples of each variety were numbered from 1 to 13. Since the amounts were limited, samples were not representative for each variety and hence results showed sample variations rather than variations among varieties. Samples originating from Scotland (3, 6, 7, 8, 10 and 11) were contemporary landraces and grown in 2010 in accordance with the local practice of only using some seaweed as fertilizer or using conventional NPK at an average of 100 kg/ha. All areas were highly humid, especially in Shetland and Hebrides with precipitation up to 1200 mm/year and had few frost days (40 days in Shetland and 34 days on the Hebrides). The annual temperature was around 7 ºC. During storage of seeds on the Hebrides, mould growth was visible. Seed samples from the same area were in previous years analyzed for plant diseases and showed both *Fusarium* and Ustilago etc. However, during oat cultivation only Ustilago was visible in the field (samples not included in present study). Hebridean samples were grown on alkaline soils, while samples from Wales (5, 12 and 13) were originally grown on acidic soils. Samples from Wales were obsolete cultivars (single-line selections and stored in genebanks). Samples from Holland (1 and 2) were contemporary cultivars originating from South America and currently on the market commonly used by commercial bulb growers. One major botanical difference between samples from Holland and the rest was that they were one-seeded and much heavier in comparison to the others, which typically had two/three seeds per floret (panicle) (*Table 2*). Information about the samples originating from Ireland (9) and the historical accession (4) are unknown. Sample 6, 7, 8 and 11 were all harvested be-

tween the end of august until the end of September. Other data concerning the oat samples, such as storage conditions, were unavailable.

Oat grains were manually de-hulled into groats and milled at ambient temperature to pass a 0.5 mm sieve in a mill from Retsch (Haan, Germany, ZM-1). Some of the grain samples were limited (around 1.4 to 2.5 g) while certain samples were larger (around 5 to 15 g). Dry matter of samples was determined by drying duplicates of about 0.1 g in 105 ºC for 16 h. Samples of grains and flours for AVA and E-vitamer analyses were stored in -20 ºC prior to analysis, while samples for β-glucan determinations were kept in room temperature until analysis.

Table 2. List of oat samples studied in the present study (Scholten, personal communication, 2012)

Sample (no.)	Variety /sample	Special character	Area or country of origin	Cropping system[1]	Fungal infections[2]	Seed w[3]
1	Soort: Evene (Japanese) haver ras: Silke	Silke	Holland	Conventional		17.0
2	Pratex	Pratex	Holland	Conventional		--
3	Orkney- agronomy institute	Black oats (landrace)	Orkney island (Scotland)	Conventional	Smut (Ustilago)	14.0
4	Strigosa subs. orcadensis		Historical accession	Unknown		--
5	S75-Wales-cultivar	S75	Wales, obsolete	Unknown		15.0
6	Hebrides STIL 7-2 double	Small oats (landrace)	Outer Hebrides (Scotland)	Seaweed or NPK	Smut	14.4
7	Shetland: Yell	Shetland aits (landrace)	Shetland islands: Yell (Scotland)	Organic		15.8
8	Hebrides North Uist 2010	Small oats (landrace)	Outer Hebrides (Scotland)	Seaweed or NPK	Smut	14.2
9	Ireland: Donegal	Black oats (landrace)	Donegal (Ireland)	Unknown		14.7
10	Orkney: West-Ray strigosa	Black oats (landrace)	Orkney islands (Scotland)	Unknown	Smut	14.4
11	Hebrides: ormadeit	Small oats (landrace)	Outer Hebrides (Scotland)	Seaweed or NPK	Smut	13.8
12	Strigosa x brevis S171 Cultivar - Wales	S171	(Wales, obsolete)	Unknown	Smut	15.7
13	Wales S76 cultivar	S76	(Wales, obsolete)	Unknown		--

[1] Cropping system in area of origin.
[2] Fungal infections as observed in field in 2010.
[3] Seed weight (per 1000 seeds) observed in 2010.

3.1.2 Content and molecular weight determination of mixed-linkage β-glucan

Content of mixed-linkage β-glucan. The analysis was performed according to McClearly & Codd (1991) (AOAC Method 995.16, AACC Method 32-23, ICC Standard Method No. 168). This method is adapted for dry cereal samples containing high levels of β-glucan, for example oat bran products. The method includes an enzyme kit supplied by Megazyme (Bray, Ireland). The method includes degradation of glucans by lichenase and β-glucosidase and the subsequent release of free glucose units is quantified by the oxidase/peroxidase reagent (GOPOD).

All thirteen samples were studied. Duplicate flour samples of 0.1 g were wet with aqueous ethanol (50 % v/v) and mixed. Sodium phosphate buffer (20 mM, pH 6.5) was added and the samples were mixed and thereafter placed in a boiling water bath and incubated for 1 min. Samples were mixed thoroughly again, incubated in a boiling water bath for 2 min and stirred. Incubation was preceded in a 50 ºC water bath for 5 min and lichenase (10U) was added and the mixture was stirred. Tubes were incubated for 1 h at 50 ºC with stirring. Sodium acetate buffer (200 mM, pH 4.0) was added and samples were mixed. Tubes were conditioned at room temperature and centrifuged. Aliquots of every sample were transferred to the bottom of three test tubes. β-Glucosidase (0.2U) in 50 mM sodium acetate buffer (pH 4.0) were added to two of the tubes, while the third tube was a reaction blank where instead 50 mM acetate buffer (pH 4.0) was added. Tubes were incubated at 50 ºC for 10 min. GOPOD reagent were added to all samples which were incubated at 50 ºC for 20 min. For absorbance measurement samples were read at 510 nm within 1 h.

β-Glucan molecular weight distribution. This method is based on size exclusion chromatography and fluorescence detection with calcofluor binding exclusively to β-glucan according to Rimsten *et al.* (2003). Extraction was performed in hot water and by the activity of thermostable α-amylase (Andersson *et al.*, 2008).

All thirteen samples were analyzed in duplicates. However, due to analytical problems some results were obtained from single samples and hence no statistical analysis was performed regarding the average mw. Ethanol (50 % v/v) was added to oat flour (0.1 g) to inactivate endogenous β-glucanase. Tubes were incubated in a boiling water bath for 15 min, cooled to room temperature and then more 50 % ethanol was added. Samples were mixed and centrifuged and supernatants were decanted. Ethanol (50 %) was added again and samples were mixed and centrifuged as before. Supernatants were decanted and tubes were stored with screw caps on in the refrigerator overnight. Later 0.28 mg/ml $CaCl_2$ in milli-Q water was added together with α-amylase (termamyl), samples were mixed and directly put in a boiling water bath for 6 h with occasional stirring. Samples were cooled to

room temperature and thoroughly mixed, followed by centrifugation. The collected supernatants were filtered (45 µm) into vials and 50 µl were injected. Molecules smaller than 10^4 are excluded from this analysis, since they are not detected. The calcofluor average molecular weight ($\bar{M}_{cf}$) was calculated according to Rimsten *et al.* (2003).

3.1.3 Content of tocopherols and tocotrienols

Twelve samples were analyzed (sample 13 was not analyzed due to limited sample amount). This analysis is based on a modified method described by Lampi & Piironen (2009). In their review they claim that cereal originated E-vitamers can be collected through a direct extraction in e.g. methanol without a prior saponification step and then detected in a HPLC system. This knowledge was utilized in the present study since both E-vitamers and AVAs were extracted by the same method.

Extraction. Oat flour in triplicates of 0.25 or 0.5 g (dependent on sample available) was mixed together with 24 ml acidified 80 % ethanol (ethanol: water: glacial acetic acid, (v/v/v), 84.2:15.7:0.1) according to a modified method from Collins & Burrows (2012). Samples were extracted 1 h in a 50 ºC water bath under occasional shaking. The samples were centrifuged in a Multifuge 3s Heraeus at 3000 rpm during 5 min and supernatants were collected and transferred into evaporator tubes. The extraction solution was evaporated in an evaporator (Vacuum controller V-855 with a multivapor P-12) at about 50 ºC in an adapted program from atmospheric pressure down to ca 20 mbar for 2 h and 10 min until dried. The residues from the evaporated samples were re-suspended in 1 ml methanol and placed in an UV sonication water bath for approximately 45 min with screw caps on or stored in refrigerator during the night before sonication. Re-suspended samples were transferred into Eppendorf tubes and were centrifuged in an Eppendorf centrifuge 5417C at 13000 rpm for 10 min. From the extract 300 µl were evaporated with nitrogen vapor. Residues were re-suspended in 100 µl heptane and transferred into HPLC vials for subsequent analysis.

HPLC analysis. An Aglient 1100 series with a straight phase HPLC column (LiChroCART 250-4) packed with Lichrospher 100 NH2, 5 µm, coupled to a guard column LiChroCART 4-4 (Merck KgaA, Darmstadt, Germany) was used to separate tocopherols and tocotrienols in the samples. The column temperature was 22 ºC. Injection volume was 20 µl and flow rate 1 ml/min. The isocratic phase used consisted of heptane: *tert*-butylmethylether: tetrahydrofuran: methanol (79:20:0.98:0.02 v/v/v/v). The compounds were detected by L-4250 fluorescence detector Varian LC9070 (Walnut Creek, CA, USA) at an excitation wavelength of 294 nm and an emission wavelength of 320 nm. Identification of compounds was based on the retention times of external synthetic standards of individual tocoph-

erols (α-TP and β-TP) and tocotrienols (α-TT3 and β-TT3) bought from Merck (Dermstadt, Germany). HPLC peaks were manually integrated with the software HP ChemStation Version 05.01. Quantification of compounds was based on standard calibration curves of α-TP and β-TP and concentrations were expressed as μg g^{-1} DM. Tocotrienols (α and β) were calculated as α-TP and β-TP equivalents, respectively, according to a review by Lampi & Piironen (2009). Peak areas of α-TT3 lay outside the α-TP standard calibration curve which was regarded as linear even at high concentrations in the present study.

3.1.4 Content of avenanthramide and free phenolic acid

Twelve samples were analyzed (sample 13 was not analyzed due to limited sample amount). This method is based on a modified version described by Dimberg & Jastrebova (2009). Prior to analysis the flour sample amount was assessed. It was found that the flour amount did not affect the results, i.e. the same values were obtained from samples originating from either 0.25 or 0.5 g flour.

Extraction. The supernatants from the centrifuged methanol mixtures from the extraction procedure described above (E-vitamer analysis) were transferred into HPLC-vials and subsequently analyzed.

HPLC analysis. An Aglient 1100 series with an UV-detector and a reversed phase C_{18} column (HP; ODS Hypersil, 5μm, 125×4 mm) at 22 ºC was used to separate AVAs and hydroxycinnamic acids in the samples. Injection volume was 10 μl and flow rate 1ml/min. The mobile phase used consisted of A: formic acid/acetonitrile/water 0.038:5:94.962 (v/v/v); B: acetonitrile. The gradient was linearly increasing from zero to 40 % B within 40 min. Avenanthramides were detected at 340±2 nm. Identification of compounds were based on the retention times of external standards of synthetic individual AVAs (1c, 1p, 1f, 2c, 2p, 2f, 2pd, 2fd, 3c, 3p and 3f) (Fagerlund *et al.*, 2009) and hydroxycinnamic acids (caffeic-, *p*-coumaric-, ferulic- and sinapic acid). Chromatographic peaks were manually integrated with the software HP ChemStation Version 05.01. Quantification of compounds was based on standard calibration curves of 2c, 2p, 2f, 2fd, caffeic-, *p*-coumaric-, ferulic- and sinapic acids, expressed as nmol g^{-1} DM. Samples spiked with 2c, 2p, 2f, 2pd, 2fd, 3p and 3f were used in a try to confirm peaks in some samples.

3.1.5 Steeping and false malting

Samples from two selected varieties of *A. strigosa*, number 1 and 2 (*Table 2*, Appendix *Figure 8* and *9*), were steeped and malted according to a modified method described by Collins & Burrows (2012).

Grain samples of each variety (duplicates of around 7 g) were heat treated in an oven at 37 ºC for 72 h and then at 65-70 ºC for 144 h, thereafter the seeds were

cooled to room temperature. Samples were subsequently steeped in Petri dishes almost completely filled with 150 ml 2 % $CaCl_2(H_2O)_2$ in tap water in an oven at 32 ºC for 18 h and later dried with paper towels in room temperature. Samples were thereafter surface sterilized by immersion in 60 ml aqueous sodium hypochlorite (1 %) for about 30 min and washed under running sterilized distillated water.

Next, grains were placed in Petri dishes with filter papers moisten by 6 ml sterilized tap water and false malted in an oven at 37 ºC for 95 h and 40 min in darkness. The rate of germination was then visually determined. Grains were defined as germinated if the sprout was visible. Samples were then dried in an oven at 37 ºC for 41 h and one of the duplicate samples were manually dehulled into groats as above. This was done as groats are ingredients in food products in contrast to grains and therefore more interesting from a food science perspective. In addition, results of native groats were obtained in the variety sample screening test and hence results from native and malted samples could be compared. However, by this experiment design the statistical evidence was decreased. On the other hand, this experiment was the first attempt to evaluate malting effects in *A. strigosa*.

Samples of native grains, steeped and malted grains and groats were subsequently milled and analyzed by the same methods as already described above for β-glucan, tocopherols, tocotrienols, AVAs and DM. The results obtained for groat samples of variety 1 and 2 in the variety sample screening experiment (see above) were included for comparisons.

3.1.6 Statistical analysis

Variations and correlations among samples were studied through One-way ANOVA analysis, General Linear Model and Turkey's pairwise comparison using the software Minitab release 11.12 (Minitab Inc., State College, PA, USA). The level of significance was set to $p<0.05$. Principal component analysis (PCA) was done using the software Unscrumbler Version 8.0 (Camo, A/S, Trondheim, Norway). All statistical analyses were based on analytical triplicate or duplicate of single sample of each variety.

4 Results

4.1 Variation among variety samples of β-glucan, tocopherols, tocotrienols and avenanthramides

β-Glucan content and molecular weight (mw) distribution. Statistical analysis showed significant difference (p=0.000) among studied samples regarding their β-glucan content which ranged from 1.9 % to 6.3 % (*Table 3*). Sample 8 had the lowest content while Sample 1 had the highest content among the samples. The average content of β-glucan among the samples was 4.7 %. The mw distribution was unimodal for all samples. One representative sample (Sample 13) is shown in *Figure 10* (Appendix), where the β-glucan population is collected in one peak around 10^9 g/mol. No statistical analysis was performed concerning the average mw, since some results were obtained from single samples. The average mw of β-glucan varied between 1.59 (Sample 8) and 2.43×10^6 g/mol (Sample 11) (*Table 3*).

Table 3. β-Glucan content and average mw of variety samples (*A. strigosa*), expressed as means of duplicates if not otherwise mentioned. Samples which do not share a letter for β-glucan content were significantly different (p=0.000)

Sample	β-glucan content (% w/w)	($\bar{M}_{cf}$) 10^6 g/mol
1	6.3^{a}	-[1]
2	5.9^{ab}	-[1]
3	4.7^{de}	-[1]
4	4.4^{ef}	2.33
5	5.0^{cd}	2.16[2]
6	4.0^{fg}	2.39
7	3.7^{g}	2.09
8	1.9^{h}	1.59
9	5.4^{bc}	2.26[2]
10	5.0^{cde}	2.37[2]
11	3.9^{g}	2.43
12	5.1^{cd}	2.37[2]
13	5.5^{bc}	2.23

[1] Samples were not analyzed.
[2] Result was calculated from single sample.

Tocopherols and tocotrienols. α-Tocotrienol was the main E-vitamer in all variety samples, except in Sample 8 where α-TP was the dominant form (*Table 4*). α-Tocotrienol and β-tocotrienol were quantified as α-TP and β-TP equivalents, respectively. Statistical analysis revealed a significant difference among samples regarding their contents of α-TT3, α-TP, β-TT3 and β-TP (p=0.000) (*Table 4*). The contents of α-TT3, α-TP, β-TT3 and β-TP varied between 7.4 (Sample 8) and 60.0 µg/g DM (Sample 10), 5.1 (Sample 7) and 15.9 µg/g DM (Sample 4), 1.3 (Sample 1) and 7.2 µg/g DM (Sample 10) and between 0.8 (Sample 7 and 10) and 3.6 µg/g DM (Sample 8) respectively. When the total sum of studied E-vitamers was taken into account a significant difference was also observed between samples (p=0.000) and the highest total sum was found in Sample 10, while Sample 7 had the lowest content (*Figure 11*). Furthermore, statistic significant correlations were found between α-TP and α-TT3 (R=0.476, p=0.005), between α-TP and β-TT3 (R=0.470, p=0.006) and between α-TT3 and β-TT3 (R=0.661, p=0.000).

Table 4. Content of measured tocopherols and tocotrienols in variety samples of *A. strigosa* expressed in µg/g DM calculated from mean values of triplicates if not otherwise mentioned. Samples which do not share a letter were significantly different (p=0.000) within each compound (column)

Sample	α-TT3[2]	α-TP	β-TT3[3]	β-TP
1	25.3^{ef}	10.2^{c}	1.3^{d}	1.5^{c}
2[1]	27.5^{cde}	8.5^{c}	1.5^{cd}	1.1^{c}
3	50.7^{abc}	12.0^{bc}	5.9^{ab}	1.4^{c}
4[1]	28.9^{de}	15.9^{a}	4.1^{bc}	1.1^{cde}
5	54.2^{ab}	11.4^{bc}	2.9^{cd}	1.2^{cd}
6	46.1^{abcd}	13.8^{ab}	3.8^{bc}	1.3^{c}
7	11.1^{fg}	5.1^{d}	1.6^{cd}	0.8^{e}
8[1]	7.4^{g}	11.0^{bc}	3.8^{bcd}	3.6^{a}
9	45.0^{abcd}	11.3^{bc}	3.6^{bcd}	2.2^{b}
10	60.0^{a}	11.0^{bc}	7.2^{a}	0.8^{de}
11	40.8^{bcde}	12.7^{abc}	3.8^{bcd}	1.4^{c}
12	29.2^{e}	11.9^{bc}	3.3^{bcd}	1.4^{c}

[1]Calculated from mean value of duplicates.
[2]Calculated as α-TP equivalents.
[3] Calculated as β-TP equivalents.

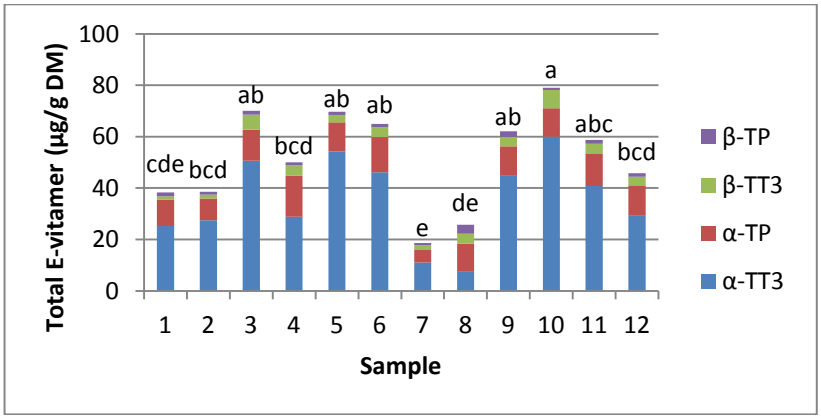

Figure 11. Total content of studied E-vitamers in the samples (µg/g DM). Samples which do not share a letter above the bars were significantly different (p=0.000).

Avenanthramides and free phenolic acids. Among AVAs the dominant form differed between samples. In most samples 2f was the dominant form, while 2c was the major AVA in Sample 2 and 4. Furthermore, 2p was the major AVA in Sample 8. Statistical analysis revealed a significant difference among samples regarding the contents of 2c, 2p, 2f and 2fd (p=0.000) (*Table 5*). The contents of 2c, 2p, 2f and 2fd varied between 11 (Sample 8) and 352 nmol/g DM (Sample 4), 19 (Sample 10) and 211 nmol/g DM (Sample 4), 7 (Sample 8) and 435 nmol/g DM (Sample 12) and between below detection limits (Sample 8) and 112 nmol/g DM (Sample 1) respectively. Statistic significant correlations were found between all major AVAs, except between 2p and 2fd (R=0.272, p=0.126) (*Table 6*). Furthermore, statistical analysis showed grouping of samples into four classes according to the total content of studied AVAs (*Figure 12*). Sample 1, 4 and 12 were all placed in the group with the highest contents, followed by 2, 3, 5, 6, 9 and 11 as middle high samples. Finally, Sample 7, 8 and 10 were grouped as the lowest samples. The highest total sum of studied AVAs where found in Sample 12 and the lowest content was found in Sample 8.

A significant difference was also evident between samples regarding caffeic-, ferulic- and sinapic acids (p=0.000) (*Table 5*). Caffeic acid was the major hydroxycinnamic acid in all samples except in Sample 8 and varied between 17 (Sample 8) and 134 nmol/g DM (Sample 7). The contents of ferulic acid were fairly similar between samples and varied between 16 (Sample 7) and 36 nmol/g DM (Sample 12). Sinapic acid was not detected in Sample 1 and 12, but ranged in the other samples between 10 (Sample 8) and 34 nmol/g DM (Sample 2). *p*-Coumaric acid was only detected in Sample 8 (81 nmol/g DM).

Table 5. Content of the avenanthramides (2c, 2p, 2f and 2fd) and hydroxycinnamic acids (caffeic-, *p*-coumaric-, ferulic- and sinapic acids) in samples expressed as nmol/g DM calculated from mean values of triplicates. Samples which do not share a letter within each column were significantly different (p=0.000)

Sample	2c	2p	2f	2fd	Peak x[1]	Peak y[1]	Caffeic acid	Ferulic acid	Sinapic acid	*p*-Coumaric acid
1	220^{b}	118^{c}	260^{c}	112^{a}	1026	305	88^{ef}	32de	N.D.[2]	N.D.
2	165^{c}	103^{c}	128de	14^{c}	252	N.D.	66^{f}	33^{d}	34^{ab}	N.D.
3	100^{d}	106^{c}	165^{d}	14^{c}	193	N.D.	63^{f}	21^{e}	25^{bcd}	N.D.
4	352^{a}	211^{a}	307^{b}	11^{c}	214	N.D.	69^{f}	29de	14^{def}	N.D.
5	64^{ef}	46de	87^{fg}	13^{c}	126	N.D.	62^{f}	23de	18^{cde}	N.D.
6	78^{def}	59^{d}	99^{efg}	12^{c}	125	N.D.	60^{f}	22de	14^{def}	N.D.
7	21^{g}	48de	65^{gh}	9^{c}	64	N.D.	134^{a}	16de	20^{ab}	N.D.
8	11^{g}	21^{f}	7^{i}	N.D.	15	N.D.	17^{g}	30de	10^{ef}	81
9	44^{fg}	36^{ef}	115^{ef}	23^{c}	81	N.D.	100^{b}	25^{c}	25^{a}	N.D.
10	16^{g}	19^{f}	29^{hi}	13^{c}	22	N.D.	82^{c}	28^{bc}	22^{ab}	N.D.
11	96de	54de	101^{efg}	15^{c}	165	N.D.	70^{cd}	31^{ab}	18^{abc}	N.D.
12	229^{b}	186^{b}	435^{a}	41^{b}	326	N.D.	62de	36^{a}	N.D.	N.D.

[1] Expressed in peak area (mAU×time)/g DM.
[2] ND – Not detected.

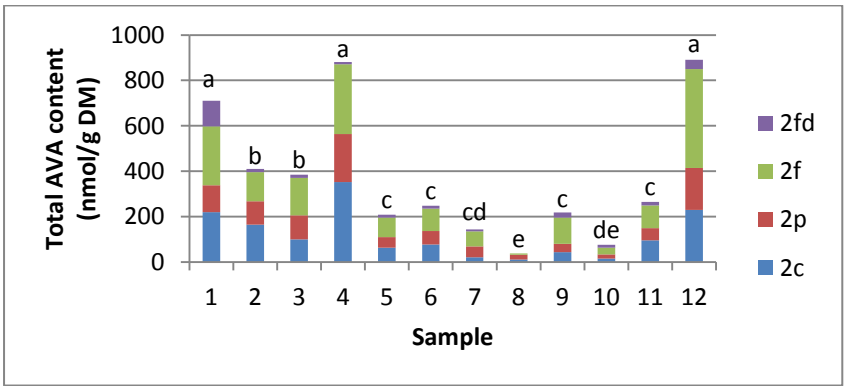

Figure 12. Total content of studied avenanthramides in samples expressed in nmol/g DM. Samples which do not share a letter above the bars were significantly different (p=0.000).

Table 6. Correlations between major avenanthramides (2c, 2p, 2f, 2pd and 2fd)

	2c	2p	2f	2pd
2p	R=0.952, p=0.000*			
2f	R=0.855, p=0.000*	R=0.920, p=0.000*		
2pd	R=0.638, p=0.000*	R=0.503, p=0.003*	R=0.443, p=0.010*	
2fd	R=0.374, p=0.032*	R=0.272, p=0.126	R=0.458, p=0.007*	R=0.808, p=0.000*

* Statistic significance (p<0.05).

HPLC chromatograms also showed some unknown peaks (*Figure 13*, Appendix). An unknown peak, peak x (*Table 5*) was evident in all samples. The retention

time of peak x was close to the retention times of 3p and 3f (25.588 and 25.549, respectively). However, samples spiked with synthetic standards of 3p and 3f revealed that peak x was obtained in between these two compounds. In addition, another unknown peak, peak y, was found but only in Sample 1. None of the reference compounds (1c, 1p, 1f, 3c, 3p and 3f) corresponded to any peak in the chromatogram. The standard peak of 1f with retention time 32.020 was close to one peak in sample chromatograms, but was not identified. This peak was found in all samples, except in Sample 12.

4.2 Effects of false malting on β-glucans, tocopherols, tocotrienols, avenanthramides and free phenolic acids

Some grains (ca 10) in one Petri dish of Sample 1 were visibly infected with moulds after malting, but were despite this analyzed. The germination rate was 0 % for Sample 1 and 2.

β-Glucan content and molecular weight distribution. In native grains the β-glucan content was 5.1 % in Sample 1 (*Table 7*). After false malting Sample 1 showed a β-glucan content of 3.8 % (75 % retained). The corresponding figures for groats were 6.3 % (native) and 5.1 % (malted) (81 % retained). The mw distribution was unimodal in native grains (native groats were not analyzed), while it was polymodal in malted grains and groats (Appendix, *Figure 14* and *15*). The β-glucan population was distributed in two or three peaks, where the smaller peaks had lower averages than the major peak. The major peak at around 10^9g/mol in native grains was retained after malting even though it was slightly relocated to the left towards lower mw. The average mw of β-glucan after malting was 1.50 and 1.71 $\times 10^6$ g/mol in grains and groats, respectively. This was lower than in native grains (2.17 $\times 10^6$ g/mol).

In native grains of Sample 2 the β-glucan content was 5.1 % (*Table 7*), while it was 3.7 % (73 % retained) in false malted grains. In native groats of Sample 2 the β-glucan content was 5.9 % and in malted groats, 4.2 % (71 % retained). The same trend as shown in Sample 1 in terms of mw distribution and appearance of mw distribution curves was also seen for Sample 2, with lower average mw in malted grains and groats (1.48 and 1.29 $\times 10^6$ g/mol respectively), than in native grains (2.23 $\times 10^6$ g/mol) (Appendix, *Figure 16* and *17*). In a similar way as for Sample 1, the β-glucan population was distributed in two or three peaks. The smaller peaks had lower averages than the major peak which was retained after malting, although relocated to the left towards lower mw compared to in native samples.

Tocopherols and tocotrienols. In both variety samples, the proportion of each E-vitamer was relatively unchanged after malting. The main E-vitamer was α-TT3 in all malted samples, followed by α-TP (*Table 7*). There was no significant differ-

ence in the content of E-vitamers between native and malted samples independent of the variety and the sample character (grain/groat). There was also no significant difference between Sample 1 and 2 regarding E-vitamers independent on if samples were native/malted and grain/groat, except for α-TT3 (p=0.033) which content was higher in Sample 2. There were however, significant differences in terms of α-TT3 (p=0.000) and α-TP (p=0.001) between grain and groat samples regardless of the variety and if samples were native/malted. This was apparent as generally groat samples had higher contents compared to grain samples.

Table 7. Effects of false malting on β-glucan, studied E-vitamers and AVAs in Sample 1 and 2. All results shown were calculated from mean values of duplicates, except results for native groats in Sample 1 and 2, which were calculated from means of triplicates regarding studied E-vitamers, avenanthramides and hydroxycinnamic acids

	Sample 1				**Sample 2**			
	Native grains	**Native groats**	**Malted grains**	**Malted groats**	**Native grains**	**Native groats**	**Malted grains**	**Malted groats**
β-glucan								
Content (% w/w)	5.1	6.3	3.8	5.1	5.1	5.9	3.7	4.2
$(\overline{M}_{cf})\ 10^6$	2.17	-[1]	1.50	1.71	2.23	-[1]	1.48	1.29
Studied E-vitamers [2]								
α-TT3	11.7	25.3	15.2	22.6	20.4	27.5	23.4	29.5
α-TP	5.2	10.2	6.5	8.6	8.6	8.5	7.2	8.7
β-TT3	1.0	1.3	0.9	1.1	1.2	1.5	1.1	1.3
β-TP	0.9	1.5	1.0	1.3	1.2	1.1	1.1	1.2
Avenanthramides[3]								
2c	147	220	138	182	119	165	199	290
2p	85	118	35	47	66	103	63	107
2f	215	260	138	189	90	128	162	226
2fd+2pd[4]	180	255	342	436	57	59	1117	1886
Peak x[5]	747	1026	2215	2466	183	252	15821	8300
Peak y[5]	248	305	905	1011	44	N.D.	3114	4815
Caffeic acid	74	88	N.D.	N.D.	69	66	N.D.	N.D.
Ferulic acid	39	32	28	60	54	33	27	20
Sinapic acid	N.D.	N.D.	N.D.	N.D.	N.D.	34	N.D.	N.D.
p-Coumaric acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[1] Samples were not analyzed.
[2] Expressed in µg/ g DM
[3]Expressed in nmol/g DM
[4] Calculated as 2fd equivalents.
[5] Expressed in peak area (mAU×time)/g DM

Avenanthramides and free phenolic acids. Sample 1 had higher contents of the main AVAs (2c, 2p and 2f) in native samples compared to in malted samples (*Table 7*). In Sample 1 there was a decrease in malted samples in comparison to native ones in terms of 2c (-6 % in grains and -17 % in groats), 2p (-59 % in grains and -

60 % in groats) and 2f (-36 % in grains and -27 % in groats). Contents were though increased in terms of 2pd+2fd (90 % in grains and 71 % in groats) and ferulic acid was almost doubled (88 %) in malted groats but decreased when comparing grains (-28 %) of Sample 1. The content of caffeic acid was decreased to below detection limits, while both *p*-coumaric and sinapic acids were not detected in any sample. In Sample 2 there was a uniform increase in malted grains and groats (*Figure 13*, Appendix) compared to native samples in terms of contents of 2c (67 % and 76 % respectively), 2f (80 % and 77 % respectively) and 2fd+2pd (19-fold and 31-fold respectively). The content of 2p were decreased in malted grains of Sample 2 (-5 %), but showed an increase in corresponding groat samples (4 %). The content of caffeic acid was compared to native samples decreased to below detection limits in malted grains and groats of Sample 2. *p*-Coumaric acid was not detected in any sample. The content of ferulic acid was decreased in malted grains and groats compared to native samples (-50 % and -39 %, respectively). Sinapic acid was only found in native groats of Sample 2 and the content decreased to below detection limits in malted groats (*Table 7*).

The interaction effect of malting and variety sample did influence the contents of all major AVAs (2c, 2p, 2f, 2pd and 2fd $p<0.01$), caffeic acid ($p=0.000$) and ferulic acid ($p=0.024$). The contents of AVAs were generally higher in native grains and groats of Sample 1 compared to Sample 2, while the contents in malted samples of Sample 2 were higher in comparison. The decrease in caffeic acid was greater in Sample 1 compared to in Sample 2. Ferulic acid was increased in malted groats in Sample 1, in contrast to Sample 2 where the content was decreased. Statistic significant differences was noted between all native and malted samples independent of the variety and the sample character (grain/groat) concerning 2p ($p=0.014$), 2pd ($p=0.003$), 2fd ($p=0.008$) and caffeic acid ($p=0.000$). Generally the content of 2p and caffeic acid were higher in native samples than in malted samples, while the content of 2pd and 2fd were higher in malted samples compared to in native ones. There was a significant difference between all grain and groat samples regardless on the variety and if native/malted concerning 2c ($p=0.012$) and 2p ($p=0.010$). This was evident since groat samples generally contained higher contents than grains.

HPLC chromatograms showed some unknown peaks (*Figure 13*, Appendix). As shown before peak x was neither 3p nor 3f and hence the peak remains unknown. This peak was increased upon malting in both Sample 1 and 2. The increase was 197 % in grains and 140 % in groats of Sample 1. In Sample 2, an 85-fold increase of peak x was found in grains and a 32-fold increase in groats. One additional peak was also increased upon malting (peak y) close to 2p, which however was not identified. The peak was found in all malted samples but was not detected in native groats of Sample 2. A 265 % increase was found in grains of Sample 1 and a

70-fold increase in grains of Sample 2. In addition, some non-separated peaks (peaks z) were also increased during malting. The interaction effect of malting and variety sample did affect the peak area of peak x and y ($p=0.000$). The increases were larger in Sample 2 compared to in Sample 1. Statistic significant differences was noted between all native and malted samples independent of the variety and the sample character (grain/groat) concerning peak x ($p=0.003$) and peak y ($p=0.001$). This was obvious since malted samples had higher peak areas than native ones.

5 Discussion

5.1 Source of variation among samples

The variation of the content of β-glucan, E-vitamers and AVAs in *A. strigosa* is not as comprehensively described as for *A. sativa*, therefore the discussion and comparison of results is based on corresponding values obtained in studies of common oats. In the present study, material available for the different variety samples differed from around 1.5 to 10 g. This may have influenced the results, as smaller samples will be more influenced by individual kernels than larger ones. The results were also obtained from replicate analysis of one single sample of each variety. A vast improvement would be to mill larger and equal amounts of oat samples to get representative flour samples of each variety and perform analysis of several batches.

The nutritive composition of oats is affected by genotypic and environmental factors (Peterson *et al.*, 2005; Shewry *et al.*, 2008; Andersson & Börjesdotter, 2011; Germeier *et al.*, 2011). The studied oat samples were cultivated in different locations and hence the environmental influence cannot be excluded as a source of variation among variety samples (*Table 2*). Some samples with the same origin from Holland (Sample 1 and 2), Orkney Islands Scotland (3 and 10) and Wales (5, 12 and 13) did indeed group together in the β-glucan content analysis (*Table 3*) and in total E-vitamers (*Figure 4*), but not in total AVAs (*Figure 12*). Other samples did not group together accordingly to origin. There also did not seem to be any grouping of samples in accordance to cropping system. Some samples were marked by fungal infections, but these samples (3, 6, 8, 10, 11 and 12) did not show any grouping in that respect, although they were not consistently significantly different in total E-vitamers (*Figure 11*). Among variety samples, Sample 8 stood out from the rest in terms of substantially lower β-glucan content and average mw (*Table 3*). Furthermore, the main E-vitamer was α-TP in contrast to α-TT3 in other samples (*Table 4*). In addition, the β-TP content was comparatively high in Sample 8. Besides this, Sample 8 was always lowest or among the lowest ones

in terms of AVAs and was the only sample where *p*-coumaric acid was detected (*Table 5*). In addition, the botanical features (Appendix, *Figure 5*) were differing from the other oat samples as groats were shorter and rounder but simultaneously more shrunken in comparison (own visual observation). Other variety groat samples were generally similar to each other; about the same length and thin. In addition, also Sample 4 did distinguish itself compared to other samples, as it had higher contents of the major AVAs (2c, 2p and 2f) (*Table 5*) and also higher contents of α-TP (*Table 4*).

β-Glucan content and molecular weight distribution. Due to analytical problems and time limit Sample 1, 2 and 3 were not analyzed. The β-glucan contents of *A. strigosa* found in this study were within the range reported by Rimsten *et al.* (2003), Chernyshova *et al.* (2007), Shewry *et al.* (2008) and Wood (2011) for common oats. The average molecular weights of *A. strigosa* were in accordance to Rimsten *et al.* (2003) and Åman *et al.* (2004), but higher than results reported by Shewry *et al.* (2008) in common oats. The unimodal molecular weight distribution of β-glucan in this study (*Figure 10*, Appendix) confirms the results reported in Åman *et al.* (2004) and Shewry *et al.* (2008). Both Shewry *et al.* (2008) and Andersson & Börjesdotter (2011) suggested that the average mw is more strongly affected by environmental factors than by genetic factors. Furthermore, the β-glucan content was more influenced by variety than by environmental factors compared to the mw according to Andersson & Börjesdotter (2011). In addition, they found a correlation between the content and the mw, which may be of importance in breeding where high contents probably also will lead to a selection for higher mw. Results obtained in the present study showed variation in the β-glucan content among samples of *A. strigosa* (*Table 3*) and hence the environmental and genotypic factors may have affected it. It would be interesting to evaluate if there is any correlation between the content and the average mw in varieties of *A. strigosa* to investigate the possibility to select varieties with wanted properties.

Tocopherols and tocotrienols. Shewry *et al.* (2008) found that the amounts were differing between cultivars, which were also seen in samples of *A. strigosa* (*Table 4*). In this study α-TT3 was the main E-vitamer in *A. strigosa*, which also was found for common oats according to the results of Peterson (1995), Bryngelsson *et al.* (2002a and 2002b) and Shewry *et al.* (2008). One exception to this was Sample 8, where instead α-TP was the largest E-vitamer, followed by α-TT3. These results were also reported for *A. sativa* by Irakli *et al.* (2012). Compared to the work of Peterson (1995) samples of *A. strigosa* had higher contents of α-TP and β-TT3 and similar contents of α-TT3 in groats compared to common oats, although the contents varied significantly in the present study. Compared to Bryngelsson *et al.* (2002a) and Shewry *et al.* (2008), the contents of both individual and total E-

vitamers were generally higher in the present study than in common oats. However, certain samples were lower in comparison.

Avenanthramides and free phenolic acids. Bryngelsson *et al.* (2002a) and Shewry *et al.* (2008) reports differences between cultivars regarding the major AVAs (2c, 2p and 2f), which also was seen for *A. strigosa* (*Table 5*). The contents of 2c, 2p and 2f were both higher and lower than previously reported for common oats in Bryngelsson *et al.* (2002a), Dimberg *et al.* (2005) and Shewry *et al.* (2008) due to the broad variation range. The contents of caffeic-, *p*-coumaric- and ferulic acid were generally higher in the present study compared to Bryngelsson *et al.* (2002a) and Dimberg *et al.* (2005), but similar to Shewry *et al.* (2008). Also Dimberg *et al.* (1996) reports variations between varieties. Differences between cultivars were suggested to be due to genetic, environmental and cultivation factors (Shewry *et al.,* 2008) which also likely explained some of the variance in the present study. Peterson *et al.* (2005) similarly declared that AVAs were the most affected compounds under the influence of environment compared to some agronomic traits and contents of protein, oil, β-glucan and E-vitamers. Shewry *et al.* (2008) found that 2c was the major AVA in most cultivars of *A. sativa*. This was only evident in Sample 1 and 2 in the present study, where instead generally 2f was the major AVA in *A. strigosa*. One explanation to this finding may be a suspected oxidation of 2c standards leading to standard curves underestimating the content as the slope was surprisingly steep (Dimberg, personal communication, 2012). Different AVA compositions in oats may also be caused by different availability of precursor compounds, different activity of the HHT enzyme and by different oxidation tendency among AVAs (Skoglund, 2008). However, the composition of AVAs in *A. strigosa* may also be different compared to the composition in *A. sativa*. Peak x was suspected to be either 3p or 3f according to retention times of standard and according to the work of Skoglund (2008) and Skoglund *et al.* (2008). Samples spiked with 3f and 3p did not confirm that the unknown peak x was either 3f or 3p, but a new AVA not previously found in studies of *A. sativa.* It may be possible that *A. strigosa* contain AVA or HCA compounds which are not present in *A. sativa* or alternatively not in the same amounts. Further studies are therefore needed to evaluate the specific profiles.

5.2 Effect of steeping and false malting

Sample 1 and 2 were chosen for malting because of larger sample amounts available compared to other samples. The malting method was adapted to AVAs according to Collins & Burrows (2012) where the conditions were claimed to stimulate an increase in AVAs. The effect of false malting was also evaluated for β-glucan and E-vitamers. False malting was successful since no kernels showed visible

sprouting. The fact that some grains in one Petri dish in Sample 1 were infected with moulds after malting may be partly explained by insufficient surface sterilization prior to false malting in combination with the high temperature used during the processes (32 and 37 ºC). Wilhelmson *et al.* (2001) reported mould growth of oat samples when germination temperature was elevated, but they also found mould growth already at 15 ºC. Furthermore, they identified e.g. *Fusarium* and *Pseudomonas* spp. in samples which were germinated at elevated temperatures.

β-Glucan content and molecular weight distribution. According to Wood (2011) depolymerization due to enzymatic activity is likely to occur during extraction. Pre-treatment with aqueous ethanol inactivates the endogenous β-glucanases before analysis and also do not seem to cause any substantial degradation (Åman *et al.*, 2004; Wood, 2011). Furthermore, Åman *et al.* (2004) stresses the importance of heat treatments before extraction to inactivate the enzymes. Endogenous enzymes like endo-β-glucanases are produced during malting, but may be inactivated by the heat treatments (Wood, 2011). Also Rimsten *et al.* (2003) emphasize the importance of inactivation of endo-β-glucanases for representative results of mw determinations. These considerations were taken into account in the present study and the method applied included these steps. In this study, the relatively well-retained content and mw may be caused by the pre-heat treatment ahead of steeping which may have inactivated some of the endo-β-glucanases. Wilhelmson *et al.* (2001) declared that a short germination period at low temperatures (72 h at 15 ºC) ended with oven drying, results in well retained β-glucan contents of oats (55-60 % of native β-glucan contents). The retained β-glucan contents in the present study were higher compared to the results of Wilhelmson *et al.* (2001) (*Table 7*). They further claim that the mw is not greatly affected in the beginning of germination and suggests that the degradation is initially slow, which implies that the retainment of β-glucan may be positively affected by shorter germination times. When germination is continued and the moisture content increased, the cell structures lose its integrity leading to increased availability for degrading enzymes of substrates. When also the temperature is elevated during malting but still within physiological temperature range, enzymatic activity is enhanced (Wilhelmson *et al.*, 2001). In a study by Hübner *et al.* (2010) they were able to retain an acceptable amount of β-glucan when shorter germination periods were applied. However, they found that the germination temperature played a minor role when it ranged between 10-20 ºC. Both the malting time and the temperature were longer and higher in the present study compared to Wilhelmson *et al.* (2001) and Hübner *et al.* (2010), however since the pre-heat treatment probably inactivated some β-glucanases the time and temperature during false malting were of less importance. According to Collins & Burrows (2012) false malting lead to an inducement of kernels into dormancy and hence a germination or sprouting phase is inhibited in

kernels. This may be of importance since the β-glucan content and the mw distribution are generally decreased as a response to germination and sprouting (Wilhelmson *et al.*, 2001; Doehlert & McMullen, 2003; Hübner *et al.* 2010). Despite the fact that the malting conditions were fairly harsh in the present study as the pre-heat treatment was relatively high and long, there was some degradation. Probably all endo-β-glucanases were not inactivated and therefore later stimulated by the conditions during false malting. This may explain the degradation which was observed. The polymodal mw distribution found for the samples was probably caused by the activity of present β-glucanases, which degraded the β-glucan polymer. Degradation has also been seen as a response to baking and in fresh pasta production with oats as an ingredient (Rimsten *et al.*, 2003; Åman *et al.*, 2004). The polymodal appearance of mw distribution (*Figure 15* and *17*, Appendix) may visualize the depolymerization of preferably smaller polymers, while the major peak was fairly intact although relocated to the left towards lower mw. This was probably attributed to the activity of endo-β-glucanases which if possible were hydrolyzing the ends of polymers rather than in the middle and as a result the retained mw was relatively intact. The degradation may also be partly caused by fungal enzymes since grains of Sample 1 showed mould growth, but it does not seem probable as only a few grains were infected.

Tocopherols and tocotrienols. At present time, there is little known about the responses of E-vitamers in oats after malting. It can though be concluded that the E-vitamers were relatively stable in *A. strigosa* after false malting (*Table 7*). This may be caused by an inhibition of degrading enzymes as an effect of the pre-heat treatment, although the activity was not studied. The main differences in contents of E-vitamers were found when grains and groats were compared and for the content of α-TT3 also the variety sample was of importance. In native groats compared to hulls there are higher contents of α-TP, α-TT3, 2c and caffeic acid in common oats (Bryngelsson *et al.*, 2002a). This was partly confirmed for *A. strigosa* when comparing groats with grains, especially in Sample1. Grain samples may be more diluted compared to groat samples, as the hull contains lower amounts. Haraldsson *et al.* (2004) found an increase in E-vitamers when barley samples were steeped in low temperatures and it seemed as the increase was temperature dependent. Responses after different malting temperatures were not evaluated in the present study, but may affect E-vitamers as suggested by Haraldsson *et al.* (2004). Further research is therefore motivated to evaluate the temperature effects and the role of false malting.

Avenanthramides and free phenolic acids. When studying HPLC chromatograms it was noted that some early peaks, caffeic- and ferulic acids, were decreased during malting in Sample 1 and 2. This was followed by a pronounced increase in the later detected peaks, 2pd and 2fd, especially noted in Sample 2 (*Figure 13*, Ap-

pendix). The mechanism for this is not known, but it may be attributed to the activity of the HHT enzyme (Skoglund *et al.,* 2008). Bryngelsson *et al.* (2003) and Matsukawa *et al.* (2000) both found an increase in AVAs when grains of oats were steeped. Furthermore, they found an increased activity of the HHT enzyme as a consequence of steeping. However, it is unknown whether the enzyme was active after the heat-pre treatment in the present study. Differences among cultivars after steeping and germination in terms of AVA content were reported by Skoglund *et al.* (2008) for common oats. They found that the contents of mainly 2p and 2f contributed to the overall increase of total AVAs, whereas the most abundant AVA, 2c, did not change significantly. In the present study, native and malted samples of *A. strigosa* had higher contents of the major AVAs compared to Skoglund *et al.* (2008). However, the AVA composition in germinated grains was dependent on the cultivar and time of germination according to Skoglund *et al.* (2008). They suggested that when germination time was elevated the compartmentalization was declining leading to an increased risk of oxidation due to enzymatic activity and this partly explained the AVA composition as different AVAs are more or less prone to oxidation. As suggested by Skoglund *et al.* (2008) the differences in AVA composition as affected by germination may partly be due to the activity of phenoloxidase which oxidizes AVAs (preferentially 2c). According to Collins & Burrows (2012) a 96 h false malting process showed an optimal AVA increase and therefore this time was applied in the present study. Bryngelsson *et al.* (2003) however practiced a shorter process and found that 2c, 2p and 2f did increase. However, the main increase of AVAs in samples of *A. strigosa* was primarily attributed to the content of 2pd and 2fd especially in Sample 2 (*Table 7*). The contents of caffeic-, *p*-coumaric- and ferulic acids also decreased as a response to steeping in *A. sativa* (Skoglund *et al.,* 2008). This was also seen for *A. strigosa*, though the contents of these HCAs were higher in native grains of Sample 1 and 2 compared to their findings. The decrease in ferulic acid as a response to germination was also found in a study by Donkor *et al.* (2012). Results found for malted groats of Sample 1 in the present study were though in contrast to the reference. The decrease of ferulic acid as an effect of AVA synthesis may be counteracted by hydrolytic release of ferulic acid from the cell wall component during steeping (Dimberg, personal communication, 2012).

This study revealed some different properties in *A. strigosa* after false malting compared to the responses in *A. sativa* after other malting methods, suggesting different functionality between the species. However, since the malting method applied seems to have a pronounced effect on the responses also the specific conditions should be a matter of consideration. In addition, because variations are likely to appear between varieties, it is possible that certain varieties may be more or less suitable for malting. At present time there is not enough evidence for sug-

gestions of suitable varieties of *A. strigosa* and therefore this is a matter for future studies. Nevertheless, these new results are an interesting base.

6 Conclusion

This primary screening of some samples of *A. strigosa* showed a significant variation concerning health supporting compounds. Some samples had contents which may add to the health benefits associated with oats, if used in food applications. Factors to consider should though be the contents of several bioactive compounds and also the responses after processing since responses may differ between samples. False malting may be a way to retain an acceptable β-glucan content and average mw, though some degradation seems to be inevitable. The composition of E-vitamers seemed to be unaffected by the malting procedure, while specific AVAs where clearly elevated in especially one of the variety samples. However, the importance of false malting specifically for AVAs needs further evaluation. Further research is needed to establish the whole spectrum of variations among varieties of *A. strigosa* to get a proper overview of its potential as a food crop. Furthermore, the possibility to alter health supporting substances is not fully evaluated and therefore more research is motivated. Malting methods which are tailored to keep and even increase health compounds found in oats are amiable since the variation in oats are pronounced and confirmed also in this study.

Although the wide use and beneficial health effects associated with oats are pronounced, oat cultivation is also connected with difficulties like fungal infections commonly leading to mycotoxin production which jeopardizes food and feed safety and produces low yields. Plant breeding heading to new varieties with elevated contents of β-glucan, E-vitamers and AVAs together with improved fungal-resistance is today a reasonable goal.

Even if there were similarities when comparing *A. strigosa* with *A. sativa*, because of the wide variation range, there were also some differences. Whether these differences are of importance or not for human nutrition and health, needs to be further clarified through human epidemiological studies to investigate any differences in bioactivity. There were however indications that certain varieties may be more suitable in food applications than others, although more data is needed. *A. strigosa* is well worth to investigate further according to the present results.

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Appendix

Variety oat samples (*A. strigosa*)

Figure 4. Groat samples of Sample 1, 2, 3 and 4 from the left.

Figure 5. Groat samples of Sample 5, 6, 7 and 8 from the left.

Figure 6. Groat samples of Sample 9, 10, 11 and 12 from the left.

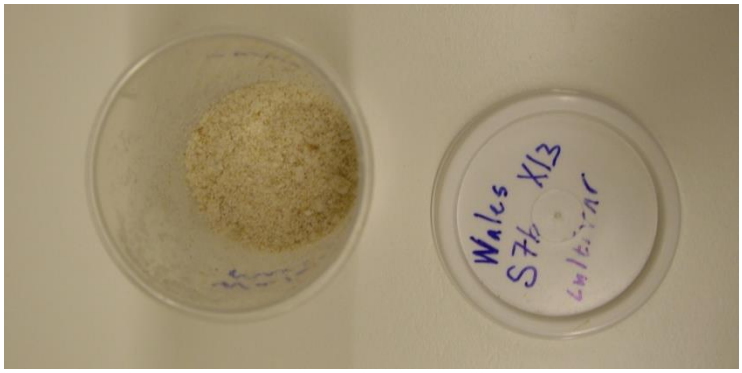

Figure 7. Flour sample (grain or groat samples not shown) from native groats of Sample 13.

Figure 8. From the left hulls, malted groats, malted grains and native grains of Sample 1.

Figure 9. From the left hulls, malted groats, malted grains and native grains of Sample 2.

Molecular weight distribution of β-glucan

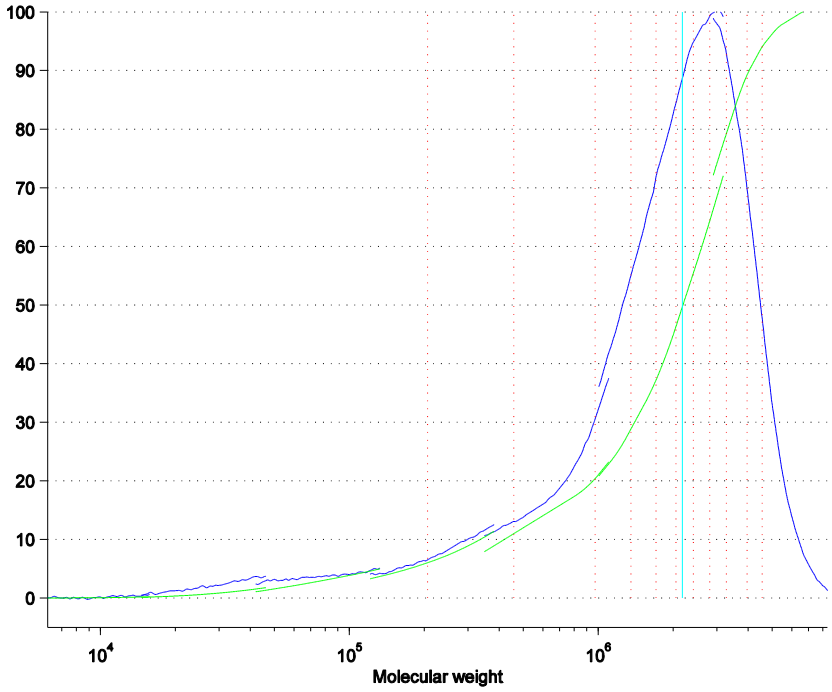

Figure 10. A representative sample of mw distribution among samples of *A. strigosa.* The mw distribution in groats of Sample 13.

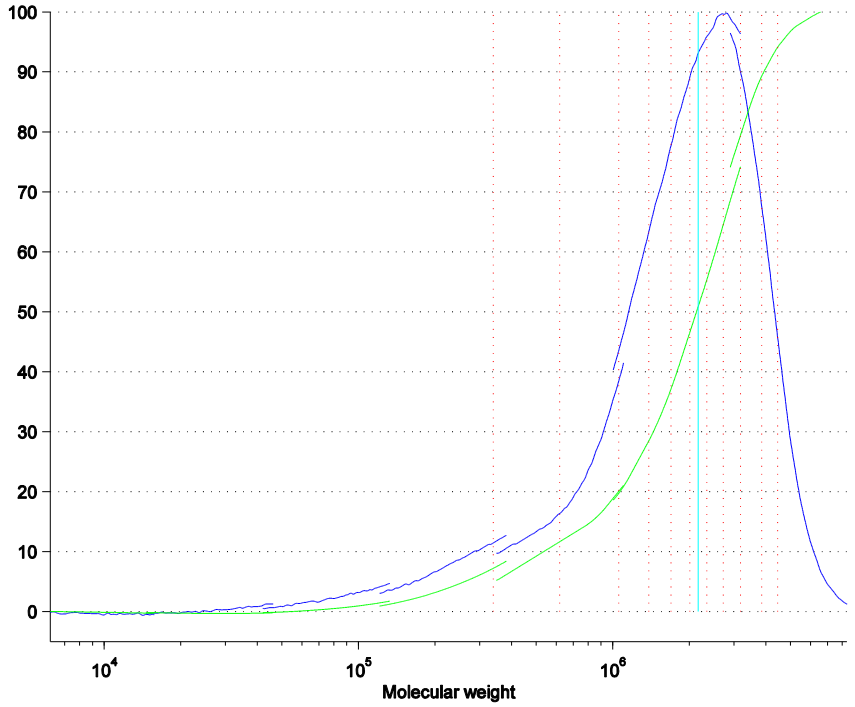

Figure 14. Molecular weight distribution of Sample 1, native grains.

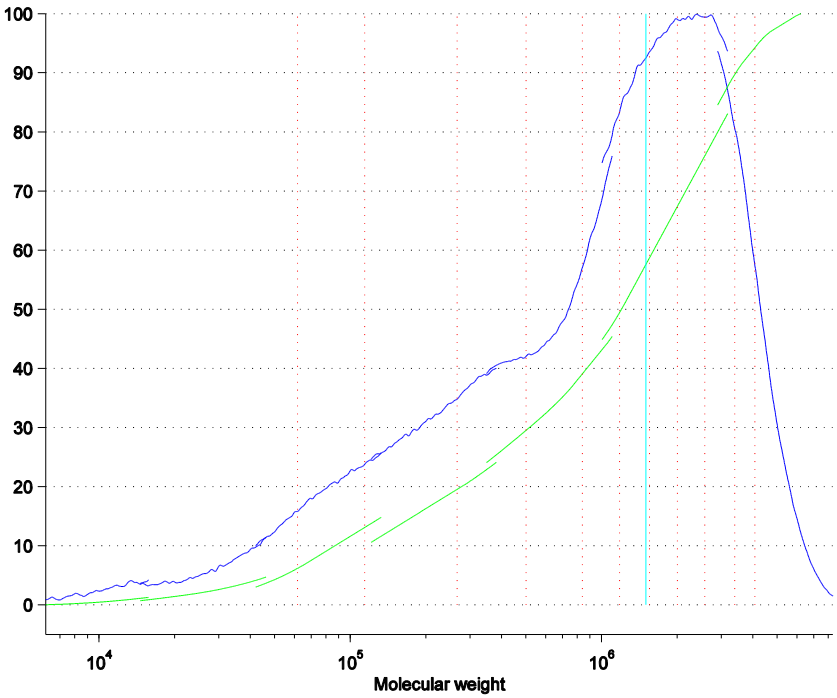

Figure 15. Molecular weight distribution of Sample 1, malted grains.

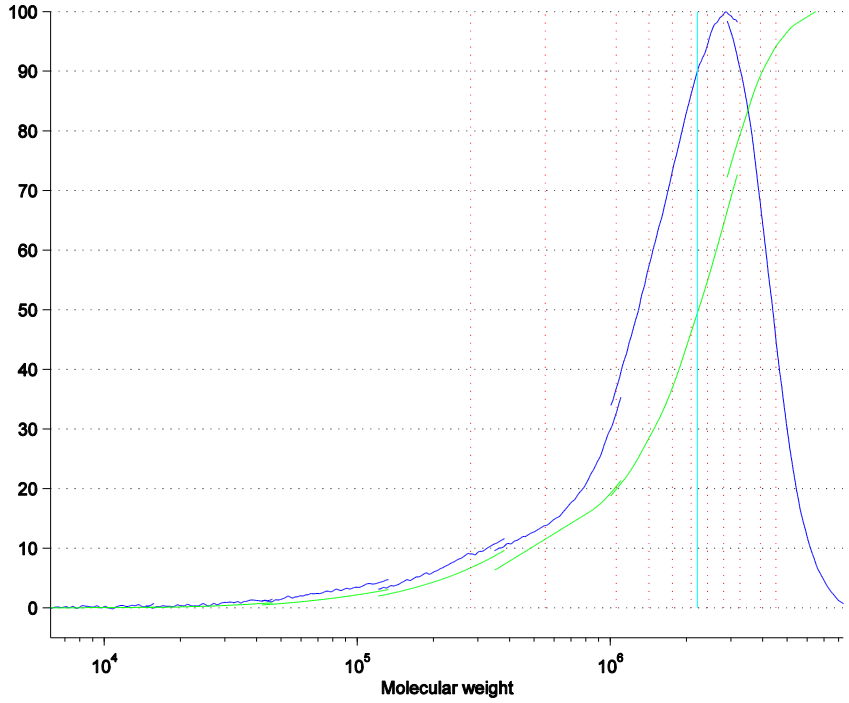

Figure 16. Molecular weight distribution of Sample 2, native grains.

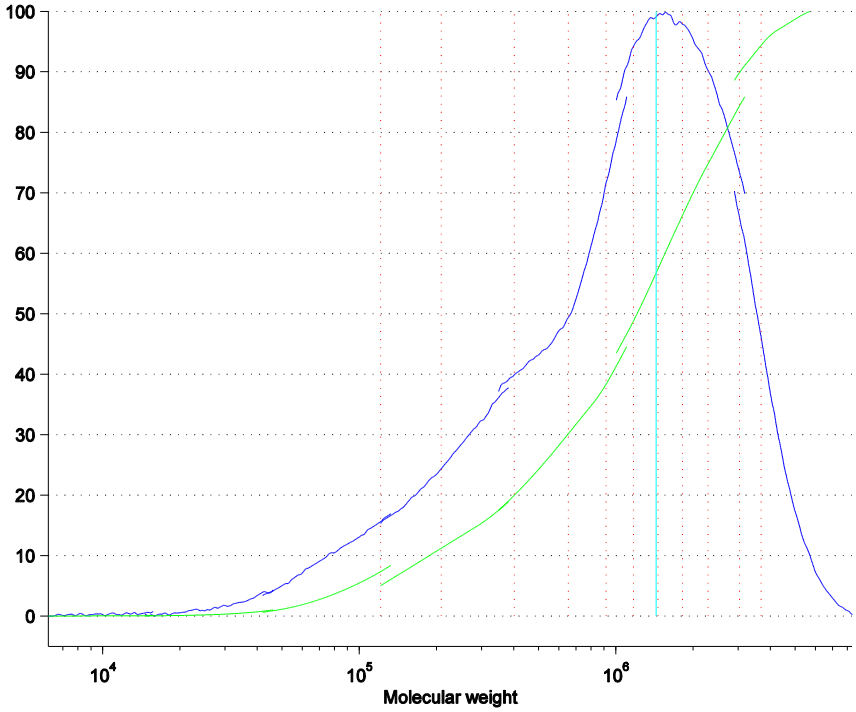

Figure 17. Molecular weight distribution of Sample 2, malted grains.

HPLC chromatogram of avenanthramides and free phenolic acids

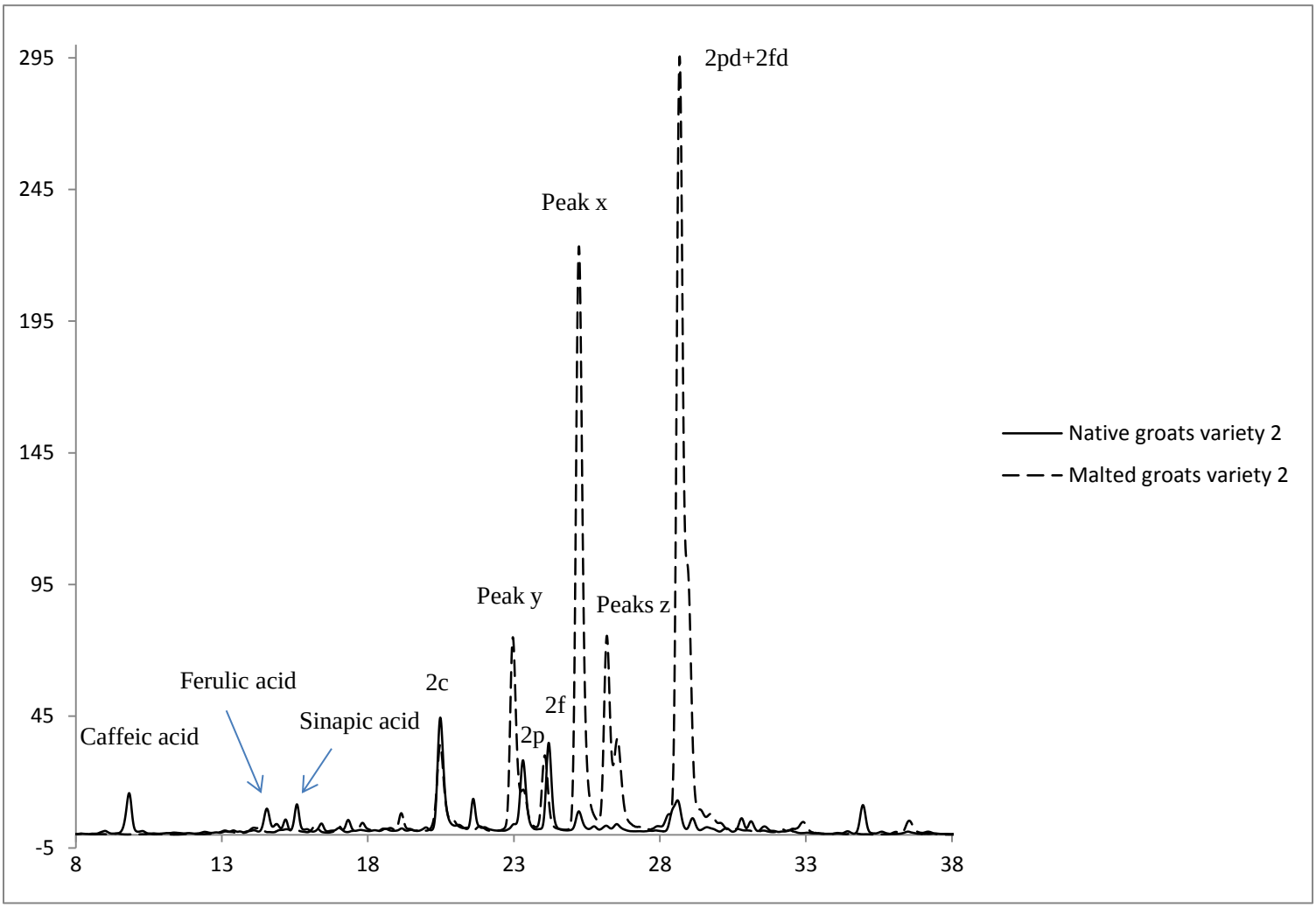

Figure 13. HPLC chromatogram of native and malted groats of variety Sample 2.

Popular scientific summary

Oats is a cereal crop which is used in many different ways, e.g. as a food, feed and as an ingredient in cosmetics. Oats is commonly used in food products such as breakfast cereals, porridges, breads and cookies. In recent years oat consumption has been highlighted due to the health benefits provided by the chemical compounds found in oat groats. These are especially the dietary fibre β-glucan, the E-vitamers tocopherols and tocotrienols and the oat specific antioxidant compounds avenanthramides. All of these compounds are thought to contribute with important health maintaining functions in the body. For example β-glucan successfully reduces the cholesterol level in the blood. High cholesterol level is one of the serious risk factors for developing heart disease. Vitamin E is essential for us, which means that we cannot synthesize it ourselves in our bodies and therefore must get it through our diet. Vitamin E is needed to protect the cells and to maintain a normal immune defense. Avenanthramides are thought to have anti-inflammatory properties which also may reduce the risk for development of heart disease. The contents of these chemical compounds are therefore highly interesting as they may be important for our health and are part of our foods.

There are different species of oats, though the most common species is *Avena sativa*. The contents of health supporting substances are known to vary between different cultivars of common oats. However, the variation in other species and varieties is less studied compared to in common oats. Scientists therefore have investigated some other species with the hope to find suitable species and cultivars with high contents. One recent study found high contents in one relative, *A. strigosa*.

It is possible to increase the contents of certain nutritive compounds in cereals through malting methods. Malting includes steeping the cereal kernel in water to start a germination process, i.e. the kernels are sprouting with the aim to build a new plant. The new plant needs energy and the nutrients are used in this process leading to a changed chemical composition within the kernel. Nutrients like proteins, fats and phenolic compounds are increased during germination. Through different malting conditions, the chemical compounds are differently affected. Malting generally lead to a decrease in the content and the size of β-glucan, while contents of avenanthramides increases as an effect. The size of the β-glucan molecule is thought to play an important role in the health benefits provided by oats. Malting conditions which retain β-glucan and increases avenanthramides simultaneously are therefore of interest. There is however still little known about the effects in vitamin E after malting.

This study aimed to investigate the differences between thirteen variety samples of *A. strigosa* regarding the contents of β-glucan, E-vitamers and avenan-

thramides. The size of β-glucan was studied in addition. Samples from two varieties were also investigated after a malting experiment to evaluate the effects on these molecules.

This study found that the contents of all investigated compounds were significantly different between the variety samples of *A. strigosa*. Especially two of the samples were different from the rest since these contained either higher or lower contents of almost all compounds. After malting there was a minor decrease in the β-glucan content and in the size, while the contents of E-vitamers were unchanged. In especially one of the samples, the increases in avenanthramides were profound. Compared to common oats there were some similarities, although also differences were observed. These differences may or may not be of importance for human nutrition and health.

This study showed that the contents of health sustaining compounds can differ between different variety samples in *A. strigosa*. Furthermore, β-glucan, E-vitamers and avenanthramides responded differently in different samples as an effect of malting. More research is therefore needed to evaluate the differences among varieties of *A. strigosa* to study its potential as a novel food crop. *A. strigosa* is well worth to investigate further according to the results found in the present study.